FDA Cardiovascular and Renal Drugs Advisory Committee
August 10, 2001

BRIEFING DOCUMENT CONCERNING TRACLEER™ (bosentan) in Pulmonary Arterial Hypertension

July 6, 2001

Actelion Pharmaceuticals Ltd.

AVAILABLE FOR PUBLIC DISCLOSURE WITHOUT REDACTION

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
AE	Adverse event
ALT (SGPT)	Alanine aminotransferase
AP	Alkaline phosphatase
AST (SGOT)	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the concentration-time curve
b.i.d.	Twice daily
BL	Baseline
Bos	Bosentan
BP	Blood pressure
bpm	Beats per minute
BREATHE	Bosentan Randomized Trial of Endothelin Antagonist Therapy for Pulmonary Hypertension (trial)
Bsep	Bile salt export pump
BUN	Blood urea nitrogen
BW	Body weight
CHF	Chronic heart failure
CIOMS	Council for International Organizations of Medical Science
CL	Confidence limit
C_{max}	Maximal concentration
CYP	Cytochrome P450 (isozyme)
DB	Double blind
ECG	Electrocardiogram
ENABLE	Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure (trial)
ET	Endothelin
ET_A, ET_B	Endothelin receptors A and B
F	Female
FDA	Food and Drug Administration
Hb	Hemoglobin
Hct	Hematocrit
HH	Marked increase (high) in laboratory variable
HIV	Human immunodeficiency virus

HTN	Hypertension
IgE	Immunoglobulin E
IgG	Immunoglobulin G
INR	International Normalized Ratio
ITT	Intent to treat (population)
i.v.	Intravenous
LFT	Liver function test
LL	Marked decrease (low) in laboratory variable
LLN	Lower limit of normal
M	Male
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MLA	Marked laboratory abnormalities
MPAP	Mean pulmonary artery pressure
MSAP	Mean systemic artery pressure
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NIH	National Institutes of Health
NYHA	New York Heart Association
OL	Open label
PAH	Pulmonary arterial hypertension
PAP	Pulmonary arterial pressure
PC	Placebo controlled
PCWP	Pulmonary capillary wedge pressure
PD	Pharmacodynamic
PHT	Pulmonary hypertension
PK	Pharmacokinetic
PPH	Primary pulmonary hypertension
PV	Plasma volume
PVR	Pulmonary vascular resistance
q.d.	Once daily
RAP	Right atrial pressure
RBC	Red blood cell
REACH	Randomized Endothelin Antagonist in CHF (trial)
SAE	Serious adverse event
SAH	Subarachnoid hemorrhage
SD	Standard deviation

SEM	Standard error of the mean
SSc/PHT	Pulmonary hypertension secondary to systemic scleroderma
t.i.d.	Three times daily
TSH	Thyroid-stimulating hormone (thyrotropin)
ULN	Upper limit of normal
WBC	White blood cell
WHO	World Heath Organization

1 EXECUTIVE SUMMARY

1.1 Introduction

Bosentan is the first orally active, non-peptide antagonist of endothelin receptors in clinical development for pulmonary arterial hypertension. It has been formulated as an oral tablet suitable for twice-daily, long-term therapy. Bosentan antagonizes binding of endothelin (ET) to both ET_A and ET_B receptors, and by blocking the actions of these receptors, it reduces the vasoconstrictive, hypertrophic and pro-fibrotic effects of ET-1. Elevated concentrations of ET-1 are strongly correlated with the severity of pulmonary hypertension in patients [1-4].

Pulmonary arterial hypertension is a progressive disease characterized by pulmonary arterial vasoconstriction, vascular remodeling, and thrombosis *in situ*, resulting in raised pulmonary vascular resistance leading to right ventricular failure and death [5]. It can occur in isolation (primary pulmonary hypertension) or secondary to systemic disease such as systemic sclerosis (scleroderma) and systemic lupus erythematosus. At the time of diagnosis, median life expectancy is from 2 to 5 years, and quality of life is poor [6, 7]. Conventional therapy includes calcium channel blockers, oral anticoagulants and diuretics, but calcium channel blockers are effective in only a small percentage of patients [8, 9]. Epoprostenol (prostacyclin) is the only currently approved treatment known to be efficacious in patients with pulmonary arterial hypertension [10], but it must be delivered by continuous infusion into a central vein via an in-dwelling catheter. In addition to the inherent problems of the delivery system and possible life-threatening complications, e.g., sepsis and thrombosis, tolerance to the compound develops over time and abrupt withdrawal elicits considerable rebound effects. An oral medication that improves symptoms and exercise capacity would provide considerable improvement to patients' quality of life.

1.2 Preclinical Pharmacology and Toxicology

By inhibiting both ET_A and ET_B receptors, bosentan antagonizes the various deleterious effects of ET-1, in particular vasoconstriction, cell proliferation and hypertrophy, and extracellular matrix formation. In animal models of pulmonary hypertension, bosentan decreases pulmonary pressure and reduces both vascular wall hypertrophy of small pulmonary arteries and right ventricular hypertrophy. Bosentan is also effective in models of pulmonary fibrosis and pulmonary inflammation. Bosentan does not modify blood pressure in situations of normal peripheral vascular resistance, it has no negative inotropic effects, it does not induce reflex tachycardia, and its chronic administration is not associated with tachyphylaxis. Like other vasodilators, bosentan causes fluid redistribution and decreases vascular permeability with resultant hemodilution, most likely responsible for the observed apparent decreases in hemoglobin concentration. Safety pharmacology studies show that bosentan has no adverse effects on the central nervous system or on gastrointestinal, respiratory or cardiac function, however urine output is transiently decreased at supra-therapeutic dosages. In toxicology and pharmacology studies, bosentan caused signs of functional cholestasis (increases in plasma bile salts in rats and dogs and increase in alkaline phosphatase), which was associated with bilirubin deposits in the liver and bile duct proliferation in dogs given high doses. No signs of hepatic cytolysis are generally noticed with the exception of transient increases in aminotransferases in dogs. In carcinogenicity studies, bosentan induced rat

thyroid and mouse liver tumors, which are among the most common tumor sites in carcinogenicity studies using pharmaceutical agents, and this same combination of tumors is found with many other drugs. Since an extensive battery of tests showed that bosentan has no genotoxic potential, these findings are considered not to represent a relevant cancer risk.

Overall, toxicology studies reveal no unusual concern for clinical use, except for the possibility that bosentan is a potential teratogen in humans.

1.3 Clinical Pharmacology

The clinical pharmacology program for bosentan included 23 studies of < 14 days duration, involving 350 healthy subjects and 221 patients, with a total of 434 receiving bosentan.

Clinical pharmacokinetics. The absolute bioavailability of bosentan was estimated to be 70% with an oral dose of 125 mg and was unchanged at steady state. Following oral administration, peak plasma bosentan concentrations were reached after approximately 3.5 hours, and the half-life was 5.4 hours. Steady state levels of bosentan are achieved within 3 to 5 days, at which time plasma concentrations are decreased by about 50% due to a 2-fold increase in clearance (most likely due to an induction of metabolizing enzymes). Pharmacokinetics are dose proportional up to 600 mg (single oral dose) and 500 mg/day (multiple doses). No clinically relevant effect of food was observed at the recommended dose of 125 mg (C_{max} increased by 22% and AUC by 10%).

Bosentan is mainly eliminated from the body by hepatic metabolism (cytochrome P450 isoenzymes CYP2C9 and CYP3A4) and subsequent biliary excretion of the metabolites. Metabolites do not contribute significantly to the pharmacodynamic effects of the parent compound (≤ 20%).

No clinically significant trend was found for bosentan exposure with regard to age, gender, race, or body weight. In patients with pulmonary arterial hypertension, intravenous (i.v.) infusion of bosentan (500 mg) led to higher plasma concentrations when compared to an historical control group of healthy subjects, caused by an almost 2-fold decrease in clearance. No pharmacokinetic data in these patients after oral administration are available, but in patients with chronic heart failure, single and multiple oral administration of bosentan resulted in both a C_{max} and an AUC that were 30% higher than values in an historical group of healthy subjects.

In patients with severe renal impairment, exposure to bosentan was not changed compared to that in healthy subjects. On the basis of single-dose pharmacokinetics, no dose adjustment is required in patients with renal impairment. Because bosentan is mainly eliminated from the body by hepatic metabolism and subsequent biliary excretion of metabolites, the effect of liver disease on bosentan's pharmacokinetics is being investigated in a specific ongoing study in patients with mild liver impairment (Child-Pugh class A).

Drug–drug interactions. Several experiments relating to possible drug–drug interactions were conducted *in vitro*, and when considered appropriate, followed up in *in vivo* studies. Results of these studies are given below.

- Although bosentan has a high extent of plasma protein binding (98%), no relevant interactions with regard to protein binding were found with digitoxin, glibenclamide (glyburide), phenytoin, tolbutamide, or warfarin.
- Bosentan is metabolized by CYP2C9 and CYP3A4, and the contribution of CYP3A4 is quantitatively more important than that of CYP2C9. Thus, the inhibition of CYP2C9 is not expected to elicit greater effects than seen with inhibition of CYP3A4.
- Inhibition of CYP3A4 with ketoconazole increased bosentan's C_{max} and AUC values 1.6- and 1.8-fold, respectively. Inhibition of CYP3A4 and possibly also P-glycoprotein by cyclosporine A increased plasma bosentan concentrations 3- to 4-fold at steady state.
- Simvastatin (substrate of CYP3A4) and losartan (substrate of CYP2C9) did not exert any influence on the pharmacokinetics of bosentan.
- Bosentan does not inhibit CYP1A2, 2C9, 2C19, 2D6, or 3A4 *in vitro* at concentrations reaching 20 to 100 μM and is not expected to increase the plasma concentrations of drugs metabolized by these isoenzymes.
- Bosentan is a mild to moderate inducer of CYP2C9, 2C19, and 3A4. In pharmacokinetic studies, bosentan treatment resulted in a 30% to 60% reduction in the systemic exposure to substrates of CYP3A4 (cyclosporine A, simvastatin and its β-hydroxy acid metabolite, glibenclamide [glyburide], and R-warfarin) and CYP2C9 (S-warfarin). No effect was demonstrated on digoxin (P-glycoprotein substrate with a narrow therapeutic window). The possibility of reduced efficacy of substrates of CYP2C9 and 3A4 should be considered when co-administered with bosentan. However, data indicate the absence of clinically relevant interaction between bosentan and warfarin.

1.4 Clinical Program

Bosentan in the treatment of pulmonary arterial hypertension was evaluated in two randomized, double-blind, placebo-controlled, multicenter studies (AC-052-351 and AC-052-352) and a total of 245 patients with severe disease (World Health Organization [WHO] functional class III–IV). Patients in Study AC-052-351 were randomized 2:1 to bosentan 125 mg b.i.d. (twice daily) or placebo, and those in AC-052-352 were equally randomized to bosentan 125 mg b.i.d., bosentan 250 mg b.i.d., or placebo. In both studies, patients were treated for 4 weeks with bosentan 62.5 mg b.i.d. or placebo, then up-titrated to the target dose (125 or 250 mg b.i.d. or placebo) for a total of up to 28 weeks. All patients were to complete Period 1 (12 weeks in AC-052-351 and 16 weeks in AC-052-352). In AC-052-351, patients continued treatment into Period 2 (up to an additional 16 weeks) until the last person entered who was not prematurely discontinued completed Period 1. In AC-052-352, only the first 48 patients enrolled were scheduled to continue into Period 2 for an additional 12 weeks treatment. Patients who completed either trial

or who were discontinued during Period 2 because of clinical deterioration were eligible to enter an open-label extension trial (AC-052-353 or AC-052-354).

In both studies, the primary endpoint was the change from baseline to the end of Period 1 (Week 12 in AC-052-351 and Week 16 in AC-052-352) in the 6-minute walk test, and the primary analysis was on the comparison between bosentan- (combined dose groups in AC-052-352) and placebo-treated patients. Main secondary endpoints included the change in dyspnea on exercise (Borg index), change in WHO functional class, time to clinical worsening, and change in central hemodynamics (AC-052-351 only). Long-term data are provided by the open-label extension of the first pivotal study in which all but one patient have received at least 1 year of bosentan treatment (125 mg b.i.d.). No data were available from the extension study of the second pivotal study as of the clinical cut-off date.

The safety of bosentan was evaluated in a wider database consisting of 11 therapeutic trials in four indications. Data from five placebo-controlled studies and two open-label studies in other indications (chronic heart failure, systemic hypertension, and subarachnoid hemorrhage) have been integrated with those from the studies in pulmonary arterial hypertension to provide a database containing 972 patients. Patients in this database not only have different indications, but also received different bosentan doses (from 100 to 2000 mg/day) for different treatment durations (ranged from 4 to 28 weeks for most studies; extension studies are ongoing). Limited safety data were contributed by seven patients with pulmonary arterial hypertension who participated in an exploratory study in which patients received an open-label, 500-mg i.v. dose of bosentan followed by oral doses of either bosentan 1000 mg b.i.d. or placebo. Two patients in this study randomized to placebo died on Day 3, and the study was terminated early. Finally, selected safety data from the still blinded study (ENABLE, n = 1613) currently being conducted in chronic heart failure have been used to support conclusions drawn from the integrated database.

The efficacy and safety findings from this large and varied database are summarized below.

1.5 Efficacy in Pulmonary Arterial Hypertension

Compared with placebo, twice daily treatment with bosentan 125 mg or 250 mg was associated with:

– Statistically significant and clinically meaningful increases in walk distance (6-minute walk test), indicating improvement in exercise capacity. The improved walk distance during treatment with bosentan was maintained for at least 32 weeks, with no evidence for tolerance.

– Consistent improvements in walk distance in all subpopulations assessed, regardless of demographics, disease characteristics, and baseline parameters (e.g., baseline walk test and hemodynamics).

– Improvement of dyspnea during exercise which was achieved with a greater walk distance, suggesting symptom relief.

– A greater proportion of patients with improvement in WHO functional class, including in the worst cases (WHO class IV).

– A significantly lower risk of clinical worsening over the 28 weeks of treatment, which was already observed at the Week-16 assessment, suggesting that bosentan may modify the course of disease.

– Significant improvements in central hemodynamic parameters (pulmonary pressures, pulmonary vascular resistance, right atrial pressure, and cardiac index) with no associated increase in heart rate (assessed with bosentan 125 mg b.i.d. only).

– Sustained benefit in patients treated for at least 1 year.

The consistent improvements in all clinical and hemodynamic parameters induced by bosentan in patients with pulmonary arterial hypertension indicate that bosentan is an effective oral therapy that can bring to these patients improved exercise capacity and relief of symptoms and may modify the course of the disease.

1.6 Effects on Hepatic Enzymes

Preclinical studies. In toxicology studies, high dosages of bosentan caused mild signs of cholestasis and in dogs, transient increases in aminotransferases. No histological evidence was found for hepatotoxicity in marmosets, rats, or dogs given daily bosentan dosages in 4-week, 6-month, and 12-month studies.

Mechanistic studies show that a likely mechanism for the liver enzyme changes after bosentan is a concentration-dependent competition by bosentan and its metabolites with the biliary elimination of bile salts, resulting in a retention of bile salts and a secondary increase in plasma aminotransferases. In contrast, there is no evidence for the formation of a reactive metabolite, an immuno-allergic mechanism, mitochondrial toxicity, or direct hepatotoxicity of bosentan or its metabolites.

Clinical studies. Treatment with bosentan is associated with an increased incidence of elevated liver aminotransferases (> 3 × upper limit of normal [ULN]). The overall incidence is about 11% and is dose related. Typically, the increase in liver aminotransferases is asymptomatic. Symptoms such as abdominal pain, nausea, vomiting, and fever were reported concomitantly in a few cases (8 of the 74 patients in placebo-controlled studies who had elevated liver aminotransferases). In about 90% of cases, the increase in liver aminotransferases appears during the first 16 weeks of treatment. The increase is gradual, and in 50% of cases, it is transient (i.e., returns to baseline or near baseline during continued bosentan treatment). Stopping treatment resulted in rapid and complete resolution of the increase, and there have been no cases with evidence for continued liver injury.

Using the Council for International Organizations of Medical Science classification scheme, most of the cases of liver injury were considered either hepatocellular or mixed (hepatocellular and cholestatic), but the mechanism(s) for the liver injury have not been clarified. Competitive inhibition of bile salt excretion could be one of the contributing mechanisms.

Assessment and management of risk. Although no case of acute liver failure was reported with bosentan treatment, the Zimmerman criteria (both liver aminotransferases and bilirubin > 3 × ULN, with little change in alkaline phosphatase) were used to estimate the risk of developing acute liver failure. In the entire database including the ongoing, still blinded ENABLE study, three patients were identified who had elevated liver aminotransferases and bilirubin > 3 × ULN, but they also had elevated alkaline phosphatase (2-3 × ULN). Although none of these patients strictly met the Zimmerman criteria, based on a conservative approach, the estimated risk of acute liver failure with bosentan was calculated to be 1/5000. This risk can be effectively managed and reduced by taking the appropriate precautionary measures. These measures include performing monthly evaluations of liver aminotransferases for the first 6 months of treatment and quarterly thereafter, establishing guidelines for dose reduction and treatment cessation, and educating both professionals and patients about the risk and management of elevated liver aminotransferases.

1.7 Effects on Hemoglobin Concentration

Preclinical studies. In toxicology studies in rats and dogs, bosentan induced mild decreases in red blood cell (RBC) parameters that were dose-dependent, maximum after a few weeks, and stabilized thereafter. As with other vasodilators, the most likely mechanism for these findings is a bosentan-induced fluid redistribution with an increase in plasma volume, resulting in relative decreases in RBC parameters. Indeed, in rats bosentan resulted in an increase in plasma volume concomitant with a decrease in hematocrit. A decrease in erythropoietin secondary to an improvement in renal blood flow could not be ruled out. There was no evidence for hemolysis, hemorrhage, bone marrow depletion, or immuno-allergic reaction in any of the studies.

Clinical studies. Treatment with bosentan was associated with a dose-related, modest decrease in hemoglobin concentration. Most of the effect was observed during the first weeks of treatment, followed by stable hemoglobin concentration during continued treatment. Overall the mean treatment-related decrease in hemoglobin concentration was about 0.8 g/dl. While more than 50% of bosentan-treated patients had a decrease by at least 1.0 g/dl (56.8% vs 29.0% with placebo), marked decreases in hemoglobin concentration were observed in 5.6% and 2.6 % of bosentan- and placebo-treated patients, respectively. In contrast, the incidence of a decrease in hemoglobin concentration to < 10.0 g/dl was similar among bosentan- and placebo-treated patients, but blood transfusions occurred in 1.8% and 1.0% of patients, respectively.

Similar to the findings in preclinical studies, the in-depth analysis of the safety database did not support hemolysis, bone marrow toxicity, or bleeding tendency as possible mechanisms behind these observations. However, the decrease in hemoglobin concentration observed among bosentan-treated patients could be explained by hemodilution. This hypothesis is based on the mechanism of action of bosentan (vasodilation and decreased vascular permeability), the preclinical observations of increased plasma volume with decreased hematocrit, and the clinical characteristics of the decrease in hemoglobin concentration.

Assessment and management of risk. Because in most cases the bosentan-related decrease in hemoglobin concentration is modest, stabilizes after the first few weeks of treatment, and is unlikely to reach levels that require blood transfusions, the risk to the patients is low. However, it

is recommended that hemoglobin concentrations be checked after 1, 3, and 6 months of treatment. This will ensure that if a marked decrease in hemoglobin concentration occurs, it will be identified and the appropriate measures taken (e.g., further evaluation to determine the cause or specific treatment, if deemed necessary based on clinical judgment).

1.8 Safety and Tolerability of Bosentan

Based on a thorough evaluation of the integrated safety database, the following conclusions emerge regarding the use of bosentan in patients with pulmonary arterial hypertension:

- Treatment with bosentan was well tolerated over a wide range of doses and in all studied indications. Among the most common adverse events reported in the safety database, those associated with bosentan treatment were flushing (placebo-subtracted incidence of 4.9%), abnormal hepatic function (3.8%), leg edema (3.3%), headache (3.0%), and anemia (2.4%), all of which appeared to be dose related.

- There was no evidence for an effect of bosentan treatment on the incidences of death or serious adverse events. The proportion of patients who had treatment prematurely discontinued due to adverse experiences was greater in patients on bosentan than in those on placebo, mainly due to elevated liver enzymes.

- Treatment with bosentan at high dosages (1000–1500 mg/day) was associated with an increased incidence of worsening heart failure in patients with severe chronic heart failure during the first month of treatment. This observation is likely related to the high starting dose and the speed of up-titration in this study. Early worsening of chronic heart failure was not reported in patients with pulmonary arterial hypertension treated with the recommended dosing regimen.

- Treatment with bosentan is associated with increased risk of liver injury, which is related to the increased incidence of elevated liver aminotransferases. Although to a lesser extent, marked decrease in hemoglobin concentration is also an associated risk.

- No relevant treatment-emergent electrocardiographic changes or changes in quantitative electrocardiographic parameters were observed in bosentan-treated patients, even at very high oral doses.

- No clinically relevant change in heart rate was observed with bosentan at any dose evaluated. Small mean decreases in blood pressures were observed that were dose dependent. However, reports of hypotension, postural hypotension, syncope, and dizziness in the overall population were more frequent on placebo. In patients with pulmonary arterial hypertension, hypotension occurred more frequently on bosentan but was generally mild and did not result in treatment discontinuation.

– Rebound effects were not observed in patients with systemic hypertension and in those with severe chronic heart failure, even after withdrawal of very high dosages. However, due to lack of experience in patients with pulmonary arterial hypertension, treatment should be stopped gradually, if possible (i.e., dose reduction to half the target dose for 3 to 7 days before full discontinuation).

The two main safety concerns that arose from the thorough evaluation of the bosentan safety database were bosentan's association with increased incidences of elevated liver aminotransferases and decreased hemoglobin concentrations. The incidences of both these abnormal laboratory findings appeared to be dose related, but the timing, severity, and potential risk to the patient differed. However, the clinical characteristics of both the abnormalities allow for appropriate management and reduction in the risk to the patient.

1.9 Dosage Recommendations

Based on the efficacy findings in patients with pulmonary arterial hypertension and the pharmacokinetic and safety findings in all clinical studies with bosentan, the dose recommendations for the treatment of pulmonary arterial hypertension are as follows:

– Initial starting dose is bosentan 62.5 mg b.i.d.

– After 4 weeks of initial treatment, the dose should be increased to 125 mg b.i.d., the maintenance dose.

No dose adjustment is required based on age, gender, race, or renal status.

An increased risk of elevated liver aminotransferases was observed in patients receiving concomitant therapy with glibenclamide (glyburide). Such patients should be followed closely, and liver function tests should be performed when initiating glibenclamide treatment. If increases in liver aminotransferases occur, alternatives to glibenclamide treatment should be considered.

Co-administration of bosentan and cyclosporine A should be used only when the potential benefits outweigh the risks, as the resulting increase in bosentan concentration may increase the risk of hypotension and/or elevated liver aminotransferases. Patients should be followed closely, and the maximum dose of bosentan should not exceed 62.5 mg b.i.d. Although there is no experience with tacrolimus, the potential exists for an interaction similar to that seen with cyclosporine A and, therefore, the same precautions should apply.

Bosentan should not be given to patients with moderate to severe liver impairment, to pregnant women, or to women who may become pregnant unless adequate contraceptive measures are taken. It is not known whether this drug is excreted in human milk, and therefore, bosentan should be discontinued when breast-feeding.

1.10 Risks / Benefits

Pulmonary arterial hypertension is a debilitating, progressive disease associated with poor quality of life, poor prognosis and few treatment options.

Benefits. In clinical studies, treatment with bosentan was associated with consistent improvements in all clinical (walk distance, WHO functional class, dyspnea during exercise, and time to clinical worsening) and hemodynamic parameters (pulmonary pressures and resistance, right atrial pressure, and cardiac index). These improvements indicate that bosentan is an effective oral therapy that can bring to these patients improved exercise capacity and relief of symptoms and may modify the course of the disease.

Risks. Treatment with bosentan was well tolerated but was associated with risks related to an increased incidence of elevated liver aminotransferases, a decrease in hemoglobin concentration, and potential teratogenic effects in the case of pregnancy.

Overall assessment. As an oral therapy, bosentan represents a significant advance in the treatment of patients with pulmonary arterial hypertension. It offers convenient treatment with considerable clinical benefit that can improve the quality of life of these patients and may modify the clinical course of the disease. The risks to patients can be reduced and effectively managed with appropriate precautionary measures, patient and physician education, and monitoring within the current treatment practices for these patients.

Overall, oral treatment with bosentan at the recommended dosages is associated with considerable benefits to patients suffering from a life-threatening disease that outweigh the risks.

2 INTRODUCTION

2.1 Pulmonary Arterial Hypertension

Pulmonary arterial hypertension is a progressive disease characterized by vasoconstriction, vascular remodeling, and thrombosis *in situ*, resulting in raised pulmonary vascular resistance leading to right ventricular failure and death [5]. Pulmonary arterial hypertension can occur in isolation (primary pulmonary hypertension) or secondary to systemic disease such as systemic sclerosis (scleroderma) and systemic lupus erythematosus. The estimated annual incidence of primary pulmonary hypertension is 1 to 2 cases per million individuals per year, and that of pulmonary hypertension related to scleroderma about 8 cases per million individuals per year [5, 11]. Conventional therapy includes high doses of calcium channel blockers, anticoagulants, and diuretics. However, calcium channel blockers are effective in only a small percentage of patients [8, 9]. The prognosis and quality of life of patients with pulmonary arterial hypertension are poor; patients have a median life expectancy of 2 to 5 years from the time of diagnosis [6, 7].

Currently, the main treatment for patients with severe disease includes the use of epoprostenol (prostacyclin), which has been shown to be efficacious in patients with pulmonary arterial hypertension [10]. However, epoprostenol must be continuously delivered using a portable pump system via an in-dwelling central vein catheter, and as a result, it is associated with numerous side effects and complications [5, 12]. Therefore, the availability of an effective and well tolerated oral treatment would be a major step forward in the treatment of these patients.

2.2 Rationale for Treatment with Bosentan

Endothelin (ET)-1, a potent endogenous vasoconstrictor, has been implicated in the pathogenesis of pulmonary arterial hypertension. In both animal models and patients with pulmonary arterial hypertension, ET-1 is elevated in plasma and lung tissue [1, 2], and concentrations in patients are strongly correlated with the severity of the disease [3, 4]. The role of ET-1 in the etiology of pulmonary arterial hypertension suggests that an endothelin antagonist may have potential therapeutic benefit in the treatment of this disease.

Bosentan is the first orally active, non-peptide antagonist of endothelin receptors in clinical development for pulmonary arterial hypertension. Bosentan antagonizes binding of endothelin to both ET_A and ET_B receptors and has been formulated as an oral tablet suitable for twice-daily, long-term therapy. By blocking the actions of both receptors, bosentan reduces the vasoconstrictive, hypertrophic and pro-fibrotic effects of ET-1. Experimental studies in animal models of pulmonary hypertension demonstrated that bosentan reduced pulmonary pressures, showed selectivity for pulmonary over systemic vasculature, and prevented both pulmonary vascular remodeling and the development of right ventricular hypertrophy. In the chronic hypoxic rat model, bosentan not only prevented the development of pulmonary hypertension but reversed established pulmonary hypertension, right ventricular hypertrophy, and vascular remodeling [13].

2.3 Organization of the Document

The New Drug Application for bosentan for the treatment of pulmonary arterial hypertension was filed with the FDA in November 2000. An FDA Advisory Committee meeting is scheduled for August 2001, and this document summarizes the key findings from the preclinical, toxicology, metabolic, pharmacokinetic and clinical NDA programs in the following sections:

Section 1	Executive Summary
Section 2	Introduction
Section 3	Preclinical Pharmacology and Toxicology
Section 4	Clinical Pharmacology
Section 5	Clinical Trial Program in Pulmonary Arterial Hypertension
Section 6	Efficacy of Bosentan in Pulmonary Arterial Hypertension
Section 7	Safety and Tolerability of Bosentan
Section 8	Effects of Bosentan on Hepatic Enzymes
Section 9	Effects of Bosentan on Hemoglobin Concentration
Section 10	Overall Safety Conclusions
Section 11	Recommended Dosages
Section 12	Risk/Benefit Evaluation

3 PRECLINICAL PHARMACOLOGY AND TOXICOLOGY

3.1 Introduction

Endothelin-1 was described in 1988 as the most potent and long-lasting vasoconstrictor substance ever characterized. ET-1 is also a potent co-mitogen and promotes fibrosis and inflammation. Endothelin plays an important role in the pathogenesis of a number of pathological situations, in particular pulmonary hypertension (PHT) and chronic heart failure (CHF). Plasma and pulmonary vascular ET-1 concentrations are increased in PHT and are strongly correlated with the severity of the disease. The two subtypes of ET receptors, ET_A and ET_B, are involved in mediating the detrimental effects of ET. Bosentan (Ro 47-0203) is a dual receptor antagonist of both ET_A and ET_B receptors.

This Section of the briefing book presents the preclinical pharmacology, animal pharmacokinetics and toxicology of bosentan. In addition, it reviews the experimental data on the potential mechanism of the increases in liver enzymes and the decreases in hemoglobin and hematocrit observed in clinical trials.

3.2 Pharmacology

3.2.1 Mechanism of Action

Bosentan is a competitive antagonist of ET binding to ET_A and ET_B receptors. In binding experiments, bosentan competes with the specific binding of [^{125}I]-labeled ET-1 on both human ET_A and ET_B receptors, with an affinity for ET_A receptors slightly higher than that for ET_B receptors. It is likely that *in vivo* bosentan is able to block both types of receptors. Bosentan is extremely specific for ET receptors.

3.2.2 Pharmacological Profile

The main pharmacological characteristics of bosentan are the following:

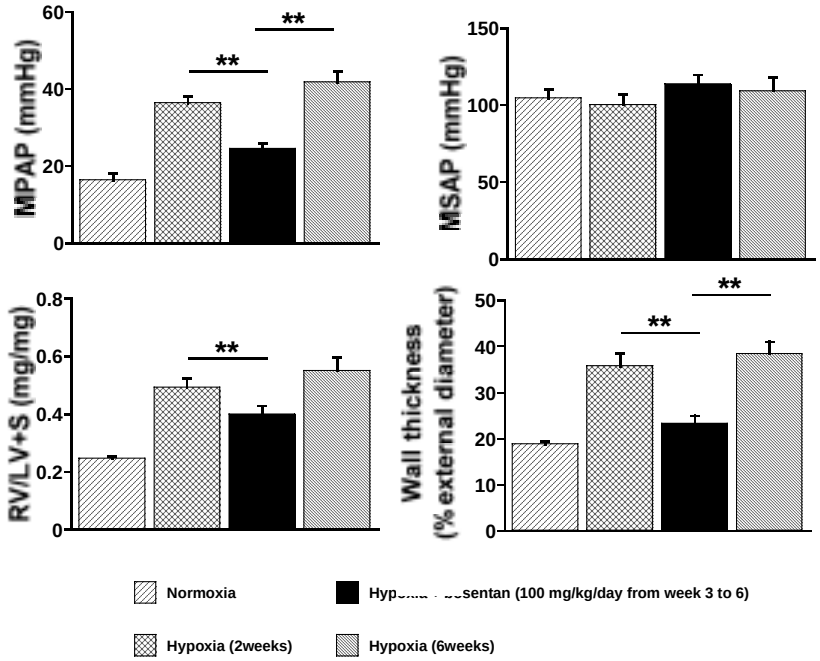
- Bosentan competitively antagonizes the contraction induced by ET peptides and inhibits the pressor effects of ET.
- Bosentan antagonizes the vasoconstrictor effects of ET-1 in human vessels where a selective ET_A receptor antagonist has little or no effect, suggesting that a combined blockade of both ET_A and ET_B receptors may be needed for full functional antagonism.
- Bosentan decreases blood pressure in pathological models of hypertension and CHF without inducing tachyphylaxis and with no reflex tachycardia. In contrast bosentan has generally no blood pressure-lowering effect in normotensive animals.
- Bosentan improves endothelial function and attenuates the production of free radicals.
- Bosentan inhibits cell proliferation and cardiac, renal and/or pulmonary fibrosis in various pathological models. Bosentan also prevents fibrosis induced by aldosterone and angiotensin II in rats, suggesting a fundamental role of ET-1 as a mediator of fibrosis.
- Bosentan increases plasma ET-1 concentrations 2- to 3-fold. No untoward consequence of ET-1 increase has been detected in any studies, in particular no tolerance and no rebound. When bosentan is given chronically, the increase in ET-1 levels is less marked after prolonged treatment than early on, suggesting a secondary decrease in ET-1 production.
- Bosentan has anti-inflammatory effects and decreases vascular permeability, leading to an increase in plasma volume and a decrease in hematocrit in normal rats and in models of increased plasma extravasation. This decrease in vascular permeability explains the efficacy of bosentan in experimental models of pulmonary edema and, most likely, the decrease in hemoglobin observed in toxicology studies.

3.2.3 Effects of Bosentan in Experimental Pulmonary Hypertension

Bosentan was tested and showed efficacy in several animal models of PHT: chronic PHT (chronic hypoxia, monocrotaline injection) and acute PHT (hypoxia-reoxygenation, extra-corporeal circulation, septic shock). Furthermore, bosentan was efficacious in animal models of pulmonary fibrosis (bleomycin) and pulmonary inflammation (oleic acid, Sephadex). The effects of bosentan in the chronic models of PHT are the following:

Bosentan decreases pulmonary artery pressure in hypoxia and monocrotaline models of chronic PHT, without decreasing systemic artery pressure. Bosentan is effective both when given in prevention and when given as intervention after development of PHT. In addition, bosentan has chronic structural beneficial effects and reduces the pulmonary arterial wall thickness of small pulmonary arteries and right cardiac hypertrophy in models of chronic PHT.

Figure 1 **Effect of bosentan on mean pulmonary artery pressure (MPAP), mean systemic artery pressure (MSAP), right ventricular hypertrophy (RV/LV+S) and pulmonary arterial wall thickness in rats with established pulmonary hypertension induced by hypoxia**



Legend: bosentan decreased pulmonary artery pressure, right ventricular hypertrophy and pulmonary arterial wall thickness, without decreasing mean systemic artery pressure. ** $p < 0.01$. Data are mean ± SEM.
RV/LV+S: right ventricular weight/left ventricle + septum weight ratio.

Overall, bosentan – given in prevention or intervention – decreases pulmonary artery pressure and improves chronic pulmonary and cardiac structural changes in animal models of PHT. Therefore, bosentan has the potential in man to improve symptomatology and exercise capacity via its hemodynamic effects, and to prevent disease progression by its effects on cardiac and vascular remodeling.

3.2.4 Effects of Metabolites of Bosentan

The main metabolites of bosentan present in human plasma are Ro 48-5033, formed by hydroxylation at the tert-butyl residue, Ro 47-8634, formed by demethylation, and the secondary metabolite Ro 64-1056, formed by a combination of these two reactions. Ro 48-5033 exhibits an inhibitory potency that is approximately 2-fold lower than bosentan, whereas Ro 47-8634 and Ro 64-1056 have a very low inhibitory potency on both ET_A and ET_B receptors. Overall, taking into account the plasma concentrations of bosentan metabolites in man and their respective inhibitory potencies, one can estimate that around 10–20% of the total ET receptor antagonistic activity of bosentan might be assigned to its metabolites.

3.2.5 Safety Pharmacology

Bosentan was studied in a battery of safety pharmacology studies at oral doses up to 300 mg/kg and intravenous (i.v.) doses up to 50 mg/kg. In these tests, bosentan showed no effect on the central nervous system and on gastrointestinal, respiratory or cardiac function. The only observation was a transient dose-dependent decrease in urine volume and electrolyte excretion in rats, seen during the first 3 hours after dosing, but not over the whole 24-hour period, except at the highest dose of 300 mg/kg. This effect is likely to be secondary to a decrease in perfusion pressure due to the vasodilatory effect of bosentan. Finally, bosentan has no effects on cardiac repolarization on rabbit Purkinje fibers.

3.3 Pharmacokinetics

The absorption, distribution, metabolism and excretion of bosentan in animals have been studied as part of its preclinical safety assessment in a variety of rodent and non-rodent species. Despite interspecies differences, data on pharmacokinetic and metabolic profiles indicate that the exposure of bosentan and its metabolites to man was covered by the study design of the toxicological program.

Bosentan was generally well absorbed after oral administration, with a bioavailability ranging from 50–70% in all species used in the pivotal toxicology studies.

Following oral administration, drug-related material was observed in several tissues that are known sites for ET receptors, including arterial vessel wall, heart, lung, kidney and adrenal gland. Levels in the brain were below the limit of detection. As expected for a drug undergoing biliary excretion, concentrations of drug-related material exceeding those in plasma were found in the liver and intestinal content.

Bosentan was predominantly eliminated by hepatic, cytochrome P450-dependent metabolism to three phase I metabolites that subsequently underwent biliary excretion. Renal excretion was negligible. Metabolism in man was mediated by cytochrome P450 isoforms 2C9 and 3A4, with a moderate affinity to these enzymes. Formation of metabolite Ro 47-8634 was exclusively CYP3A4 dependent, whereas both CYP isoforms contributed to the formation of metabolites Ro 48-5033 and Ro 64-1056. The majority of bosentan-related material was excreted within 3-5 days.

After multiple dose administration, an initial time-dependent decrease in plasma exposure was observed in several animal species, accompanied by a parallel increase in concentration and activity of microsomal proteins. In primary human hepatocyte cultures, bosentan caused mild to moderate enzymatic induction. This was confirmed in several clinical studies with a variety of substrates for both CYP2C9 (S-warfarin) and 3A4 (simvastatin, cyclosporine A, R-warfarin).

Inhibition experiments *in vitro* revealed a low potential for bosentan to inhibit CYP-mediated metabolism of other drugs. Among all CYP isoforms tested, the most pronounced inhibition was on CYP2C9 activity, with a competitive inhibition mode and a K_i of 22 μM. Overall, all IC_{50} and K_i values were far above the observed plasma concentrations at the clinical dose of 125 mg b.i.d. As anticipated from these *in vitro* data, co-administration of bosentan with drugs that were either

substrates of CYP2C9 (S-warfarin) or CYP3A4 (simvastatin, cyclosporine A) did not lead to elevated plasma concentrations of these drugs in the clinical drug-drug interaction studies.

3.4 Toxicology

The toxicology program for bosentan included acute, subchronic, and chronic studies with oral and/or i.v. administration; reproductive toxicology studies; mutagenicity studies; carcinogenicity testing; and special studies including local tolerance, skin irritation, eye irritation, antigenicity, and phototoxicity assays; metabolic studies, and several mechanistic studies. Toxicokinetic evaluations were performed in most of the toxicology studies.

3.4.1 Acute Toxicology Studies

Bosentan had a low order of acute toxicity. The highest non-lethal doses were in the 125 to 250 mg/kg range by the i.v. and intraperitoneal routes of administration, 1000 mg/kg or more by the subcutaneous route and 2000 to > 4000 mg/kg by the oral route.

3.4.2 Multiple-dose Oral Toxicity Studies

3.4.2.1 Studies in Rats

Three repeated-dose toxicity studies of up to 6 months duration were conducted in rats. There was no substantial toxicity observed in any of the rat studies. In the 4-week oral gavage study, bosentan was generally well tolerated. In female rats, slight decreases in red blood cell (RBC) parameters (within normal limits) were evident at all doses. In male rats, increased thyroxine and a trend for increased rT3 and thyroid stimulating hormone were observed. At necropsy, organ weight changes included increased thyroid weights in male rats at all doses, increased kidney weights in mid- and high-dose males and increased liver weights in mid- and high-dose males and females. In the 4-week oral (admix) study, bosentan was well tolerated. In the 6-month oral (admix) study in rats, bosentan was well tolerated. Clinical signs were limited to stertorous breathing at the mid dose and high dose along with some histological changes suggestive of local irritation in the nasal cavity. In this study, bosentan induced a significant but small increase in alkaline phosphatase at the highest dose, with no significant changes in aminotransferases, but mildly increased liver weights (see Section 3.5). There was no histopathological evidence of hepatotoxicity in any of the repeated-dose studies in rats.

3.4.2.2 Studies in Dogs

Four repeated-dose toxicity studies of up to 12 months duration were conducted in dogs. In 4-week studies in dogs, bosentan did not result in significant toxicity. Red blood cell parameters were slightly decreased. Clinical chemistry revealed transient increases in alanine aminotransferase (ALT) and alkaline phosphatase at 500 and 1000 mg/kg/day, with a maximal parallel increase at 2 weeks and a much lesser increase at 4 weeks of treatment. Organ weight changes included increased liver weights and for females increased kidney, thymus, adrenal and decreased ovary weights. Histopathologically, liver changes included minimal to slight bile duct proliferation in both sexes and in females, minimally increased single cell necrosis/granuloma were seen at both doses. In the 6-month oral (capsule) toxicity study in dogs, bosentan was generally well tolerated. The only compound-related findings occurring at the high dose

consisted of minimal decreases in RBC parameters, a minimal increase in alkaline phosphatase, a moderate increase in liver weights and a low-grade periacinar hepatocellular hypertrophy. In a 12-month oral (capsule) study in dogs, bosentan treatment was also generally well tolerated. Slightly decreased RBC parameters in high-dose dogs were observed. A minimal increase in alkaline phosphatase at the mid and high dose and a marked increase in serum bile salts at the high dose were observed. At necropsy, liver and kidney weights were increased in mid- and high-dose male dogs. Histopathologically, mid-dose dogs showed an increased yellow pigment and vacuolation in the gallbladder epithelium and at the high dose marginal signs of cholestasis, increased mucus secretion, yellow pigment and vacuolation in gallbladder epithelium, and increased yellow pigment in the kidney were observed. Two high-dose dogs had dissimilar episodes of impaired general condition, considered unlikely to be treatment related. Transient increases in reticulocytes and – in one dog – increases in aminotransferase and moderate interstitial fibrosis in the kidney were associated in both dogs with increased fibrinogen, which could suggest bacterial infection. Another dog had slight to moderate interstitial fibrosis in the kidney, considered unlikely to be treatment related.

3.4.3 Reproductive Toxicity Studies

In the fertility (Segment I) studies in rats with oral and i.v. administration, no effects were observed on mating performance or fertility, nor was there any adverse effect on the development of the preimplantation embryo or on implantation. There were no changes in sperm count, motility or viability or on testes weights.

In a combined Segment II and III embryo-fetotoxicity and pre- and post-natal study in rats, bosentan showed dose-dependent teratogenic effects that included variations in the arteries originating from the aorta, agenesis of the soft palate, and craniofacial bone abnormalities. In addition, poor post-natal survival was observed. A study conducted with a litter exchange design indicated that *in utero* effects (e.g., craniofacial abnormalities) were responsible for the poor survival of pups.

In contrast to the rat, bosentan was not teratogenic in the rabbit (up to 1500 mg/kg/day), which was confirmed in an additional high-dose (1500 mg/kg/day) study. A toxicokinetic study in rabbits revealed that the exposure to circulating bosentan in rabbits at a given dose is approximately 20-fold lower as compared to rats, which may explain the lack of teratogenicity in the rabbit.

Overall, bosentan was teratogenic in rats (but not in rabbits) at exposure levels that could possibly be achieved in humans; thus, the compound should be considered to be a potential teratogen in humans. The similarity of malformations between bosentan and those observed in ET-1 knockout mice and with other ET receptor antagonists indicate that teratogenicity is a class effect for these drugs.

3.4.4 Mutagenicity Studies

The mutagenic and clastogenic potential of bosentan were evaluated in a comprehensive battery of tests *in vitro* and *in vivo*. There was no evidence for any mutagenic or clastogenic activity with bosentan.

3.4.5 Carcinogenicity Studies

Two 2-year carcinogenicity studies were conducted with bosentan in CD-1 mice and Wistar rats along with appropriate range-finding and toxicokinetic studies. A statistically significant increase in the combined incidence of hepatocellular adenomas and carcinomas in male mice (at 450, 2000, 4500 mg/kg/day) and in the combined incidence of thyroid follicular cell adenomas and carcinomas in male rats (at 3000 mg/kg/day) were found. Using the pre-specified statistical analytical plan, no other rat tumor was considered to be related to bosentan treatment. In mice, adenomas of the colon were observed in one upper mid-dose and two high-dose male mice and two high-dose female mice, and none in the controls. This tumor incidence is statistically significant by the pre-specified trend test, but not on a pairwise comparison. These tumors are benign and, because of their relatively low incidence, they were considered not related to treatment.

3.4.5.1 Mice

A 2-year oral (dietary admix) carcinogenicity study was conducted with bosentan in CD-1 mice at doses of 0, 100, 450, 2000, and 4500 mg/kg/day. There was no increase in mortality. At necropsy, there was a dose- and treatment-related increase in liver weights in male and female mice. An increased incidence of hepatic masses was observed in male mice treated with bosentan at dose levels of 450, 2000, and 4500 mg/kg/day. Histopathologically, a statistically significant moderate increase in the combined incidence of hepatocellular adenomas and carcinomas was observed in male mice at dose levels of 450, 2000 and 4500 mg/kg/day. There was no increase in the incidence of tumors in female mice or at sites other than liver in male mice that were considered to be related to treatment with bosentan.

3.4.5.2 Rats

A 2-year oral (pelleted admix) carcinogenicity study was conducted in Wistar rats with bosentan administered at dosages of 0, 125, 500, 2000 and 3000 mg/kg/day. Pelleted diet was chosen because, in contrast to powdered diet, it did not result in local irritation in the nasal cavity in preliminary dose range-finding studies. There was no increase in mortality at any dose. After 104 weeks, there was a treatment-related significant increase in the combined incidence of thyroid follicular cell adenomas and carcinomas in male rats at the high dose of 3000 mg/kg/day. There was no significant increase in the incidence of tumors in female rats or at sites other than thyroid gland in male rats.

3.4.5.3 Conclusions

With respect to the rodent tumor findings, mice are particularly susceptible to the formation of liver tumors when treated with microsomal enzyme inducers such as bosentan (bosentan was shown to be a microsomal enzyme inducer in mice and dogs). Likewise, rats – in particular males – are susceptible to the development of thyroid follicular tumors secondary to thyroid hormone imbalance (bosentan was shown to cause thyroid hormonal imbalance in rats). Rat thyroid and mouse liver are among the most common tumor sites in carcinogenicity studies with pharmaceutical agents and since an extensive battery of tests showed that bosentan has no genotoxic potential, these findings are considered not to represent a relevant cancer risk.

3.4.5.4 Testicular Findings in Bosentan-treated Rats

In the 2-year carcinogenicity study in rats, an increase in the incidence of slight to minimal testicular tubular atrophy was observed in the treated groups as compared to the controls. This was considered not to be treatment related because of the minimal degree of severity, the lack of dose-response relationship, the severity score of testicular tubular atrophy which was not increased in the treated groups as compared to controls, and the fact that the increase in testicular tubular atrophy occurred bilaterally and, to a somewhat greater extent, unilaterally, whereas drug-induced testicular effects would be expected to occur bilaterally. In the other studies, with the exception of two males in the mid-dose but not the high-dose group of the 6-month study, no other testicular tubular atrophy was found. In various fertility studies, fertility was normal and sperm parameters (motility and counts), testis and epididymal weights and histopathology were normal.

In conclusion, the data indicate that the testicular atrophy seen in the 2-year carcinogenicity study is not treatment related. The other studies, as well as the absence of abnormality in sperm parameters after bosentan administration of up to 6 months, support this conclusion.

3.4.6 Special Studies

Special studies conducted with bosentan included evaluations for immunotoxicity, primary skin and eye irritation, and phototoxic potential.

Immunotoxicity studies. Immunotoxicity studies showed that bosentan is weakly immunogenic in guinea-pigs (with, but not without adjuvant), but not in mice. In a 12-month toxicity study in dogs, antibodies specific for bosentan were not detected and there was no increase in total IgG.

Primary skin and eye irritation. Bosentan was not irritant to rabbit eye or skin.

Phototoxic potential. The phototoxic potential of bosentan *in vitro* showed a phototoxic potential with UVA but not UVB irradiation. However, i*n vivo,* there was no evidence for phototoxicity in hairless rats.

3.4.7 Overall Conclusions

There is a good correlation between the findings in the non-clinical toxicology studies with bosentan and the effects observed in humans. There is a wide margin of safety between clinical exposures and exposures that result in significant toxicity in the toxicology studies. Concerning carcinogenicity, rat thyroid and mouse liver are among the most common tumor sites in carcinogenicity studies with pharmaceutical agents and this combination of tumors is found with many other drugs. Since an extensive battery of tests showed that bosentan has no genotoxic potential, these findings are considered not to represent a relevant cancer risk. The findings in the non-clinical toxicology studies do not raise any unusual concern for clinical safety except for the teratogenic potential of this class of drug.

3.5 Preclinical Data on Bosentan-induced Changes in Liver Enzymes

Dose-dependent increases in liver aminotransferases have been observed in clinical studies using bosentan. This Section presents the experimental data relative to the effects of bosentan on the liver in toxicology studies and the potential mechanism of the increases in liver enzymes.

3.5.1 Observations Related to the Liver in Toxicology Studies

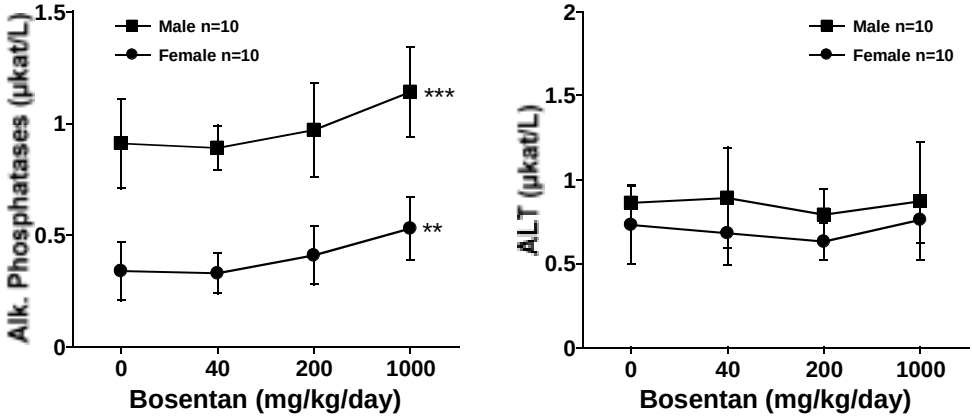
3.5.1.1 Primates

In a 4-week study in marmosets, there was no change in aminotransferases with the exception of a small increase in ALT at the mid dose of 80 mg/kg/day in males but not at the high dose of 500 mg/kg/day. The small change in the mid-dose males was considered not related to treatment. A slight increase in isocitrate dehydrogenase and increased liver weight was observed at the high dose of 500 mg/kg. There was no histological evidence for hepatotoxicity.

3.5.1.2 Rats

In the pivotal 6-month rat toxicology study, bosentan induced a significant but small increase in alkaline phosphatase, without significant changes in aminotransferases (Figure 2). Mildly increased liver weights were noted in this study at 1000 mg/kg/day and in the 4-week oral gavage study at the highest dose of 2000 mg/kg. There was no histopathological evidence of hepatotoxicity in any of the repeated-dose studies in rats.

Figure 2 Effect of bosentan on alkaline phosphatases (AP) and alanine aminotransferase (ALT) in the 6-month rat toxicology study



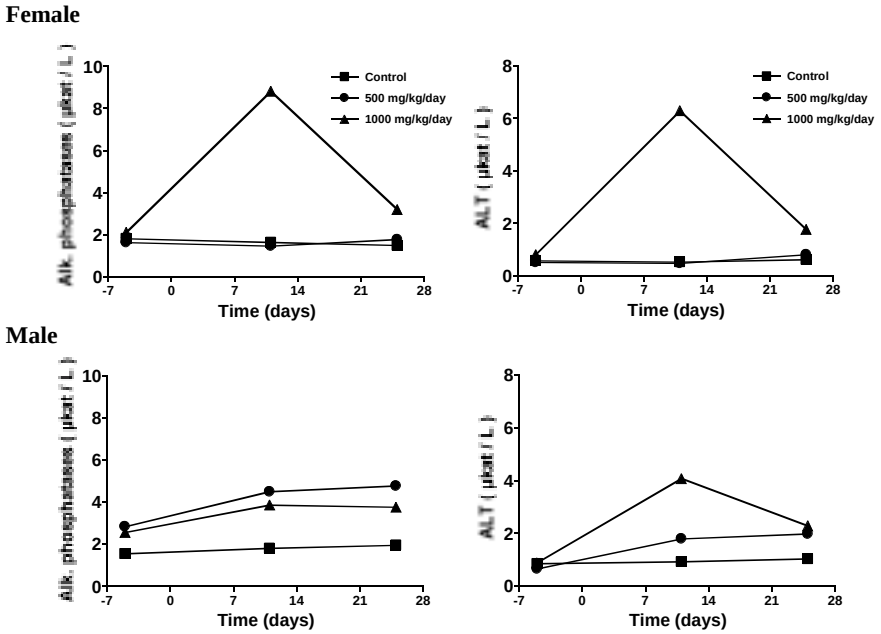
Legend: At 6 months, bosentan (1000 mg/kg/day) induces a significant increase in AP. There is no significant increase in ALT. ** $p < 0.01$, ***$p < 0.001$ vs control. Data are mean ± SD.

3.5.1.3 Dogs

In the 4-week study, increased ALT and alkaline phosphatase along with moderate increases in liver weights were observed at 500 and 1000 mg/kg. The increases in ALT and alkaline

phosphatase were transient, with a maximal parallel increase at 2 weeks and a much smaller increase at 4 weeks of treatment (Figure 3). Histologically, at 500 and 1000 mg/kg, minimal to slight bile duct proliferation was observed in males and females, and minimally increased single cell necrosis/granuloma was observed in the liver of high-dose females.

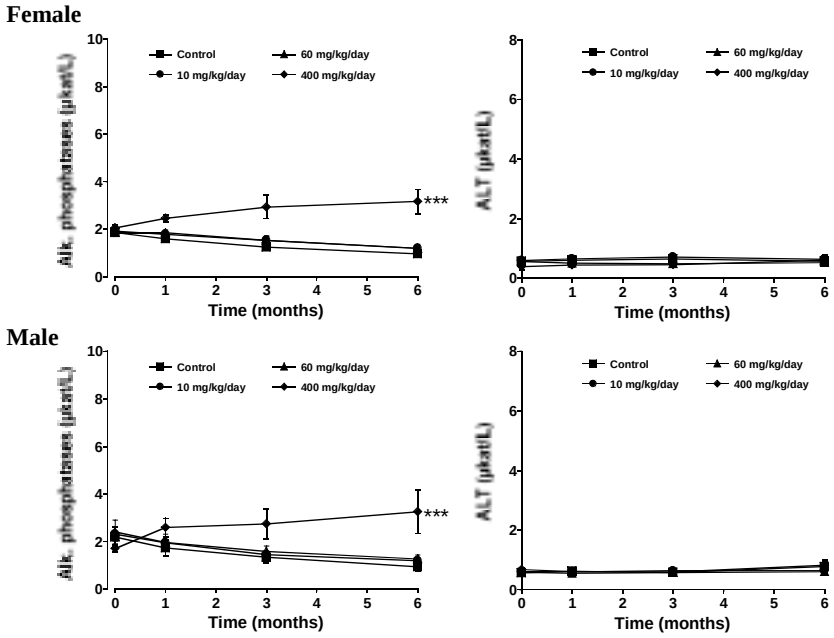
Figure 3 Effect of bosentan on alkaline phosphatases (AP) and alanine aminotransferase (ALT) in the 4-week dog toxicology study



Legend: Bosentan (500 and 1000 mg/kg/day) induces a parallel and marked increase in AP and ALT after 2 weeks of treatment. These changes are transient and at 4 weeks there a much smaller increase in AP and ALT. n = 2 per group.

In the 6-month dog study, small but statistically significant increases in alkaline phosphatase but no changes in ALT (Figure 4) were observed. Increased liver weights and a low-grade periacinar hypertrophy were observed at the high dose of 400 mg/kg, but no signs of cytolysis or centrolobular hepatocellular necrosis.

Figure 4 **Effect of bosentan on alkaline phosphatases (AP) and alanine aminotransferase (ALT) in the 6-month dog toxicology study**



Legend: Bosentan (400 mg/kg/day) induces a moderate increase in AP, comparable to the residual increase after 4 weeks of treatment in the 4-week study. ALT are not significantly modified. *** $p < 0.001$ vs control. Data are mean ± SEM.

Similarly, in the 12-month dog study, a small increase in alkaline phosphatase at 180 and 500 mg/kg was observed. This was associated with a marked increase in serum bile salts concentration. Mean ALT levels were not significantly increased. In one high-dose dog only, there was a marked but transient increase in ALT in the context of a complex disease. Increased liver weights were noted at 180 and 500 mg/kg. Histologically, increased yellow pigment and vacuolation in gallbladder epithelium were observed at 180 and 500 mg/kg/day and mild signs of cholestasis including bilirubin deposits in hepatocytes and canaliculi were observed at 500 mg/kg. There were no signs of hepatocellular necrosis.

3.5.2 Mechanistic Studies

3.5.2.1 Competition with Bile Salts Elimination is a Likely Mechanism for the Clinical Observations of Dose-dependent Increases in Aminotransferases

Several lines of evidence show that bosentan inhibits bile salt elimination in a dose-dependent manner, causing a functional cholestasis with an accumulation of bile salts, which may cause hepatocyte leakage or be cytotoxic to hepatocytes at high concentrations, leading to release of aminotransferases.

Indeed, the following effects of bosentan have been observed:

– bosentan decreases the biliary excretion of bile salts in dogs;

– bosentan increases serum bile salt concentration in rats and dogs;

– bosentan increases bile flow in rats and dogs;

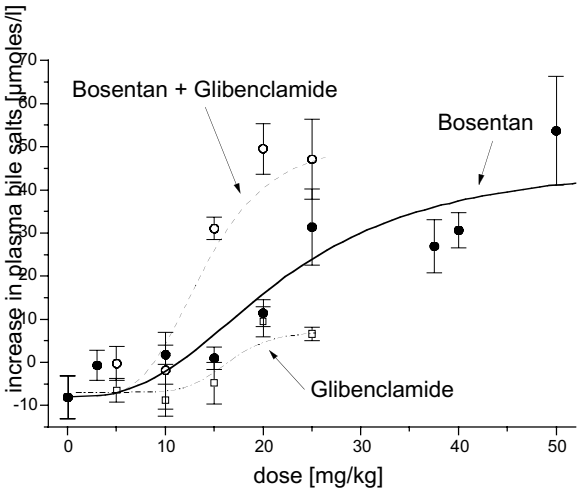
– bosentan (and its minor metabolite Ro 47-8634) compete in a dose-dependent fashion with bile salt transport at the canalicular plasma membrane by inhibiting the bile salt export pump Bsep (formerly "sister of P-glycoprotein");

– glibenclamide (glyburide) also inhibits Bsep. The combination of glibenclamide and bosentan potentiates the effect of bosentan for increasing plasma bile salt concentrations in rats.

Bosentan decreases biliary excretion of bile salts. In dogs treated for 12 months with high doses of bosentan (180 and 500 mg/kg/day), analysis of bile sampled at the end of the study revealed markedly decreased biliary concentrations of bile salts.

Bosentan increases serum bile salt concentration in rats and dogs. After i.v. injection, bosentan (10–50 mg/kg) in rats increases bile salt concentration in plasma (Figure 5). In the 12-month dog toxicology study, serum bile salts were measured and were found to be markedly increased by bosentan in a dose-dependent fashion.

Figure 5 Effect of bosentan on plasma concentrations of bile salts in normal rats

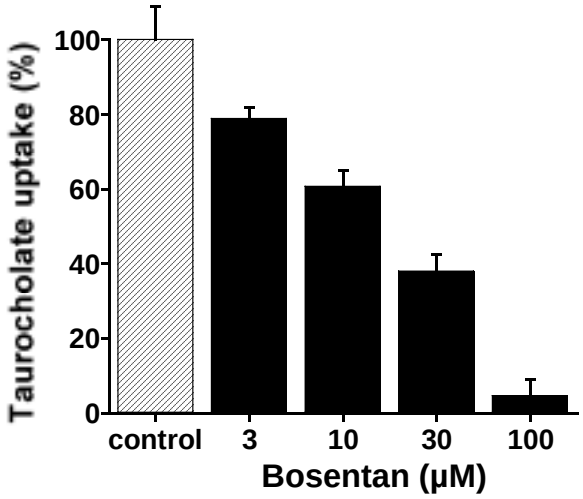


Legend: Intravenous injection of bosentan in rats increases bile salt concentration in plasma in a dose-dependent fashion at 10 min after injection. Glibenclamide has a similar effect. The combination of bosentan and glibenclamide has a synergistic effect with a greater increase in plasma bile salt concentration. Data are mean ± SEM.

Bosentan increases bile flow. Bile duct-cannulated dogs were treated orally for 4 weeks with bosentan at 20 or 500 mg/kg/day. Bosentan increased bile flow by 20% at 20 mg/kg/day and by 200–300% at 500 mg/kg/day. Thus, bosentan induces a functional cholestasis but not an obstructive cholestasis.

Bosentan competes with bile salt elimination. In rat canalicular liver plasma membrane vesicles, bosentan inhibited the ATP-dependent ^{3}H-taurocholate transport, with an IC_{50} of 10–20 µM (Figure 6).

Figure 6 Effect of bosentan on bile salt transport in canalicular liver plasma membrane vesicles



Legend: Bosentan inhibited in a concentration-dependent fashion the ATP-dependent ^{3}H-taurocholate transport through rat canalicular liver membranes. Data are mean ± SEM.

To evaluate the active transport system involved in the excretion of bile salts and in the inhibition by bosentan and metabolites of their elimination, the rat Bsep was expressed in baculovirus-infected Sf9 insect cells. Bosentan competitively inhibited ATP-dependent taurocholate transport in this recombinant system, with a K_i of 12 µM. Among the three metabolites of bosentan, Ro 47-8634 exhibited a similar inhibitory effect with an IC_{50} comparable to that of bosentan. The major metabolite Ro 48-5033 and Ro 64-1056 had no significant effect.

Glibenclamide (glyburide) potentiates the effect of bosentan. Like bosentan, glibenclamide (glyburide) also competes with bile salt elimination, in contrast to other antidiabetic drugs such as metformin and the sulfonylureas, tolbutamide, chlorpropamide and glicazide, which did not interfere *in vitro* with bile acid transport. *In vivo*, glibenclamide also increased serum bile salt concentration in rats. The combination of bosentan and glibenclamide (glyburide) led to a potentiation of the increase of bile salts in plasma (see Figure 5).

3.5.2.2 There Is No Evidence for Formation of Reactive Metabolites from Bosentan

The initial step in idiosyncratic drug toxicity in man is often due to activation of an intrinsically unreactive drug to a reactive metabolite by drug-metabolizing enzymes. Typical examples of reactive metabolites are epoxides, arene oxides, acyl glucuronides and Michael acceptor systems. The reactive metabolite subsequently binds covalently to cellular macromolecules, triggering a cascade of events finally leading to drug toxicity. Prominent examples of drugs leading to formation of reactive metabolites comprise troglitazone, tienilic acid, acetaminophen and carbamazepine. Concerning bosentan, its metabolic pathways do not suggest the potential formation of any reactive metabolites, and furthermore there is no indication of any biological response suggestive of the presence of reactive metabolites. Indeed, bosentan has no cytotoxic effect on human hepatocytes, no genotoxic potential (even in the presence of metabolic activation systems), and does not induce antibody formation.

Therefore, there are no biological observations suggesting the presence of reactive intermediates.

3.5.2.3 There Is No Evidence for Direct Cytotoxicity of Bosentan or Its Metabolites

Bosentan and its metabolites (0–750 μg/ml) were tested on human hepatocytes to assess their potential cytotoxic effects.

– In a test assessing lysosomal function (neutral red uptake), no effect was observed at concentrations below 200 μg/ml.

– In a test assessing cell integrity (LDH release), no effect was observed at concentrations below 400 μg/ml.

The peak plasma concentration in man treated with bosentan 125 mg b.i.d. is around 0.8 μg/ml. It is therefore unlikely that direct cytotoxicity is involved in humans treated with therapeutic doses of bosentan.

3.5.2.4 There Is No Evidence for an Immuno-allergic Mechanism

In toxicology studies there was:

– no increase in eosinophils,

– no increase in antibody level (total IgG) over 12 months of administration of bosentan (500 mg/kg) in dogs,

– no specific anti-bosentan antibodies in serum after a 6-month administration of bosentan (500 mg/kg) in dogs,

– no signs of lymphocyte infiltration or inflammation in the liver.

3.5.2.5 There Is No Evidence for Mitochondrial Toxicity

Bosentan and its metabolites (0–750 μg/ml) were tested on human hepatocytes to assess their potential effects on mitochondrial function (MTT reduction). The data show that no effect was

observed at concentrations below 400 µg/ml for any of the four compounds. Again, these concentrations are significantly higher than the estimated free hepatic concentrations of bosentan or its metabolites.

3.5.3 Conclusions

The concentration-dependent competition of bosentan and metabolites with bile salt excretion is the most likely explanation for the clinical characteristics of the elevations of serum aminotransferases:

– their dose-dependency,

– their reversibility,

– the frequent association with increases in alkaline phosphatase and bile salts,

– the potentiation by glibenclamide (glyburide).

There are no indications that the other mechanisms can explain the increases in aminotransferases.

3.6 Preclinical Data on Bosentan-induced Decreases in Red Blood Cell Parameters

In clinical studies, bosentan treatment was associated with a dose-related decrease in hemoglobin concentration and hematocrit. This Section presents the experimental data relative to the effects of bosentan on RBC parameters in toxicology studies and the potential mechanism of these changes.

3.6.1 Decreases in Red Blood Cell Parameters in Toxicology Studies with Bosentan

Decreases in hemoglobin and hematocrit were seen in the three long-term toxicology studies in rats (6 months) and dogs (6- and 12-month studies). The decrease in hemoglobin/hematocrit is mild, dose-dependent, maximum after a few weeks, and reaches a plateau after a maximum of 3 months (Figure 7).

In rats, after 6 months of treatment, mean hematocrit decreased in female rats treated with bosentan 200 mg/kg/day and 1000 mg/kg/day by 6.5%, associated with a significantly decrease in reticulocytes.

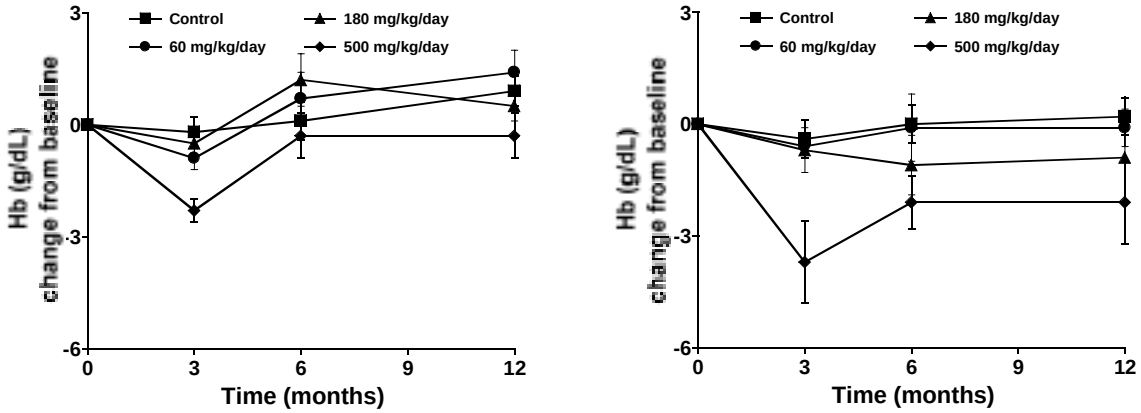
In dogs treated for 6 months with bosentan, mean hematocrit decreased by 4.5% (60 mg/kg/day) and 13.6% (400 mg/kg/day), with similar decreases in hemoglobin. No decrease was seen at 10 mg/kg/day. Reticulocytes and total bilirubin tended to decrease at 60 and 400 mg/kg/day.

In the 12-month dog toxicity study, the decrease in hematocrit was of the same order of magnitude as in the 6-month study after 6 months of treatment (−11.1% in males treated with 500 mg/kg/day), but the decrease was slightly less (−6.7 %) after 12 months (Figure 7). At 3 months and beyond, the decrease in hemoglobin/hematocrit was associated with decreases in albumin concentration of −6% (males) to −11% (females). Total bilirubin and reticulocytes tended to decrease. Thus, the overall pattern was very similar to that seen in clinical trials with

bosentan, with a maximal decrease after a few weeks and a lesser decrease after long-term treatment, and an associated decrease in albumin.

Figure 7 Changes in hemoglobin (Hb) after chronic bosentan treatment in males (left) and females (right) in the 12-month dog toxicity study

Males Females



Legend: Bosentan (180 and 500 mg/kg/day) induces decreases in hemoglobin with a maximal effect at 3 months and a smaller effect at 6 and 12 months. Data are mean ± SEM.

3.6.2 Mechanistic Studies

Several lines of evidence show that bosentan induces fluid redistribution and relative hemodilution with secondary decreases in RBC parameters. There is no evidence for hemolysis, hemorrhage or bone marrow depletion.

3.6.2.1 Bosentan Induces Fluid Redistribution and Relative Hemodilution

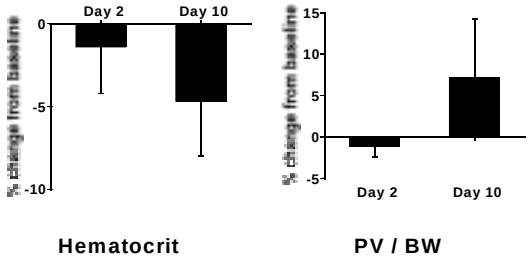
The most likely mechanism for the decrease in hemoglobin/hematocrit after bosentan is a decrease of the transcapillary exudation of fluid, resulting in an increase in plasma volume and hemodilution.

Endothelin-1, like other vasoconstrictors, decreases plasma volume by increasing transcapillary exudation of fluid, mainly to the lungs, and to a lesser extent of albumin. The resultant effects are a decrease in plasma volume, hemoconcentration and an increase in hematocrit after ET-1 injection. This effect of ET-1 might explain why patients with CHF, and with PHT, actually have an increased plasma viscosity and increased hematocrit.

Bosentan decreases albumin extravasation and prevents the reduction in plasma volume induced by ET-1 or in other pathological models. Bosentan also decreases vascular permeability,

increases plasma volume and decreases hematocrit in normal rats. Indeed, 10-day administration of bosentan (100 mg/kg/day *per os*) in normal rats significantly increased plasma volume (measured by the Evans blue dye method) corrected for body weight and decreased heamtocrit (Figure 8).

Figure 8 Effect of bosentan on hematocrit (Hct) and plasma volume (PV) corrected for body weight (BW) in normal rats



Legend: Bosentan (10 mg/kg i.v., then 100 mg/kg/day by oral gavage for 10 days) increases plasma volume (as measured by Evans blue dye method) and decreases hematocrit. Data are mean ± SEM.

3.6.2.2 Bosentan Does Not Decrease Erythropoietin in Normal Dogs

In the 12-month toxicology study in dogs, bosentan had no effect on erythropoietin concentrations. However, an absence of effect in normal dogs does not eliminate the possibility that bosentan, by improving renal perfusion, could reduce abnormally elevated erythropoietin levels in patients. Serum levels of erythropoietin are abnormally elevated in situations of renal vasoconstriction and renal hypoxia. Since bosentan improves renal blood flow in animal models of heart failure, bosentan could also decrease or normalize erythropoietin concentrations in diseased patients. Erythropoietin concentrations are also decreased in rats by angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists.

3.6.2.3 There Is No Evidence for Bone Marrow Aplasia

Bone marrow smears did not reveal any abnormalities in the three chronic toxicology studies in rats and dogs. In addition, the decrease in hemoglobin/hematocrit did not progress with time, but stabilized after a few weeks (see Figure 7), dismissing bone marrow aplasia. Therefore, there is no evidence for central bone marrow abnormality.

3.6.2.4 There Is No Evidence for Bleeding Tendency

There was no evidence for hemorrhage in any of the toxicology studies. In addition, there was no evidence for any bleeding-associated anemia. In particular, there were no signs of microcytosis or iron deficiency.

3.6.2.5 There Is No Evidence for Hemolysis

In the oral subchronic and chronic toxicity studies in rats and dogs, there was no evidence of hemolysis. Indeed, there were no signs of RBC destruction, no signs of accelerated RBC regeneration and no signs of an immuno-allergic reaction.

No signs of red blood cell destruction:

- No increase in serum total bilirubin concentrations (in contrast, bilirubin is either unchanged, or decreased as in female dogs treated at high doses in the 6- and 12-month toxicology studies).
- No increase in bilirubin and urobilinogen concentrations in urine in the various studies.

No signs of accelerated red blood cell regeneration:

- The 'anemia' is 'normocytic' (mean corpuscular volume [MCV] is unchanged in all studies).
- There is no increase in RBC size variability (no anisocytosis).
- Bone marrow composition does not reveal abnormal stimulation.
- There are no increases in mean reticulocyte counts. In contrast, mean reticulocytes at scheduled determinations were found to be unchanged or decreased in all toxicology studies where they were measured (one 4-week dog study, two 4-week rat studies, one 6-month rat study, one 6-month dog study, one 12-month dog study). However, two individual dogs treated with the highest dose of bosentan in the 12-month dog toxicology study experienced transient increases in reticulocytes, associated with clinical symptoms. These two dogs showed a dissimilar pattern of periods of poor general condition during the study. In the first dog, reticulocytes increased at a single time point as an isolated reversible event (Day 92) but the clinical symptoms (sedation, apathy) developed after 247 days. In the second dog, a clinical syndrome developed with signs of anemia, progressive increase in reticulocytes, and an elevation in fibrinogen, which could suggest an infection. Eosinophils remained between 2 and 4%, there was no increase in IgG and no anti-bosentan antibodies.

No signs of an immuno-allergic reaction:

- No IgG increase
- No anti-bosentan antibodies
- No increase in blood eosinophils in the various toxicology studies. In the 6-month rat study, there was a relative increase in bone marrow eosinophils, (associated with a relative decrease

in lymphocyte counts), whereas the eosinophils in the peripheral circulation tended to be decreased at all time points during treatment period.

3.6.3 Conclusion

In conclusion, the effect of bosentan on fluid redistribution and increase in plasma volume can explain:

– the dose-dependent decrease of hemoglobin, hematocrit, and RBCs seen in the various toxicology studies,

– the time course for the decrease in hemoglobin/hematocrit, and

– the associated decreases in reticulocytes, albumin and bilirubin concentrations.

Additionally, a decrease in erythropoietin secondary to an improvement in renal blood flow could not be ruled out.

4 CLINICAL PHARMACOLOGY

The clinical pharmacology program for bosentan included 23 studies involving 350 healthy subjects and 221 patients. The dose ranges investigated were 10–750 mg (single dose) by the i.v. route and 3–2400 mg (single dose) and 100–1000 mg/day (multiple doses) by the oral route. Eight studies were conducted to establish the pharmacokinetic, pharmacodynamic, and safety profile of bosentan in healthy subjects and in some special patient populations. Six studies investigated the potential for drug–drug interactions and nine studies were of an exploratory nature in various indications. Of the latter, two are considered relevant for this application (one in pulmonary arterial hypertension and one in heart failure).

4.1 Clinical Pharmacokinetics

Bioavailability, absorption, and food effect. The pharmacokinetics of bosentan were characterized using a number of formulations: an oral suspension, a lyophilisate for i.v. infusion, an early tablet formulation at different strengths, and the intended market formulation at different strengths. The pharmacokinetic studies conducted with the different formulations allow extrapolation from results obtained with the suspension and the early tablet formulation to the intended market formulation. The absolute bioavailability is estimated to be 70% at an oral dose of 125 mg and is unchanged at steady state. Bosentan is well absorbed following oral administration; peak plasma concentrations are reached after approximately 3.5 hours. Pharmacokinetics are dose proportional up to 600 mg (single dose) and 500 mg/day (multiple doses). No clinically relevant effect of food was observed at the recommended dose of 125 mg (C_{max} increased by 22% and AUC by 10%).

Distribution. Bosentan has a volume of distribution of 17 l after single and 30 l after multiple-dose administration (parameters determined after an i.v. dose of 250 mg). Bosentan is strongly bound to plasma proteins (98%), primarily to albumin, and does not penetrate into erythrocytes.

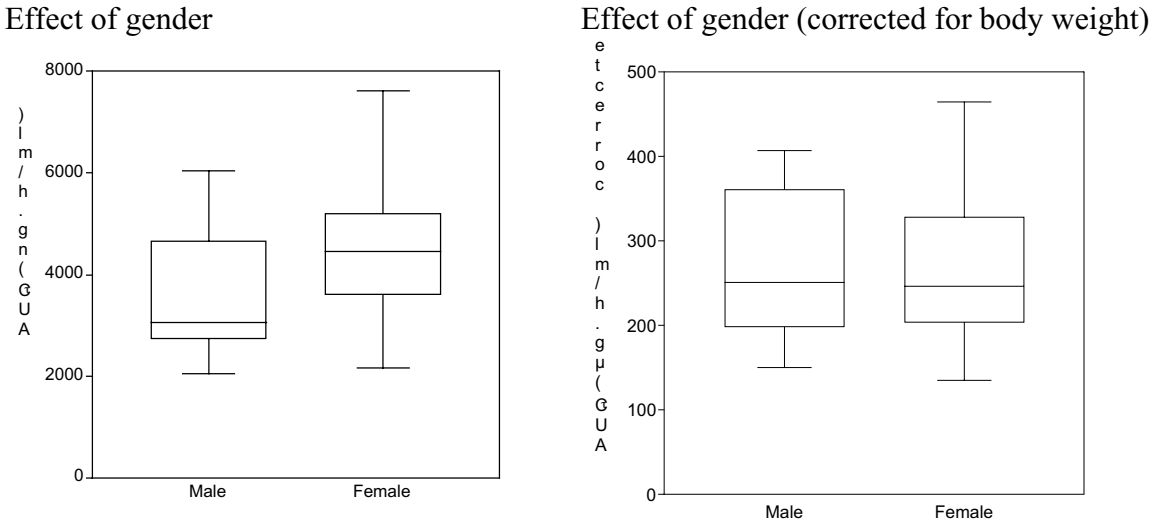
Metabolism and excretion. The clearance after an i.v. dose of 250 mg is 8.7 l/h. The half-life after a single oral dose of 125 mg is 5.4 hours. Steady-state levels of bosentan are achieved within 3–5 days after multiple dosing, at which time plasma concentrations are decreased by about 50% due to a 2-fold increase in clearance (17.4 l/h). The decrease in plasma concentration is most likely due to induction of metabolizing enzymes. At steady state the elimination half-life is virtually unchanged. Bosentan is mainly eliminated from the body by hepatic metabolism and subsequent biliary excretion of the metabolites. After i.v. administration, only 5% of the administered dose was recovered in urine (1% as unchanged drug), whereas more than 90% was excreted in feces (3.7% as unchanged drug). Following an oral dose, approximately 30% unchanged bosentan was excreted into feces, probably representing unabsorbed material. Bosentan is metabolized to three metabolites by the cytochrome P450 isoenzymes CYP2C9 and CYP3A4. One of these metabolites, Ro 48-5033, is pharmacologically active but present in low concentrations relative to bosentan. It contributes a maximum of 20% of the total response following administration of bosentan

Renal impairment. Following a single oral dose of 125 mg bosentan in patients with severe renal impairment (creatinine clearance between 15 and 30 ml/min), exposure to bosentan was not changed compared to that in healthy subjects (creatinine clearance > 80 ml/min). Although exposure to the three metabolites was 2-fold greater in renally impaired patients as compared to healthy subjects, plasma concentrations of all metabolites remained low compared to those of bosentan. On the basis of single-dose pharmacokinetics, no dose adjustment is required in patients with renal impairment. It is not expected that bosentan can be removed by dialysis because of its high extent of plasma protein binding and molecular weight.

Hepatic impairment. Because bosentan is mainly eliminated by hepatic metabolism and subsequent biliary excretion of metabolites, the drug should not be administered to patients with moderate/severe liver dysfunction. A specific study to assess the effect of mild liver impairment (Child-Pugh class A) on the single- and multiple-dose pharmacokinetics of bosentan is ongoing.

Demographic factors. Retrospective analysis of available data from studies in healthy subjects and from a study in patients with subarachnoid hemorrhage (SAH) exposed to bosentan shows that age, gender, race, and body weight have no clinically relevant influence on the AUC corrected for dose. When compared to male subjects, exposure to bosentan tended to be higher in female subjects. This appears to be solely related to the differences in body weight as shown in Figure 9.

Figure 9 Bosentan pharmacokinetics and gender



Pharmacokinetics in target population. In patients with pulmonary arterial hypertension, i.v. infusion of a high dose of bosentan (500 mg) led to higher plasma concentrations when compared to an historical control of healthy subjects, caused by an almost 2-fold lower clearance. No pharmacokinetic data are available in patients with pulmonary arterial hypertension after oral

administration. Due to similar pathophysiological characteristics, it is reasonable to expect that the pharmacokinetics of bosentan in the target population are comparable to those in patients with CHF. In CHF patients, bosentan levels after i.v. infusion were increased compared to those in healthy controls. A population pharmacokinetic analysis for CHF patients revealed a relationship between baseline cardiac index and both clearance and volume of distribution at steady state, i.e., a low cardiac index resulted in a reduced volume of distribution and clearance and greater exposure. After multiple oral doses of bosentan 500 mg b.i.d. in patients with advanced CHF, the AUC was 30% higher than in an historical group of healthy subjects. The decrease in plasma concentrations seen in healthy subjects upon multiple oral dosing was also observed in CHF patients. In analogy to the findings in CHF patients, the plasma concentrations of bosentan in patients with pulmonary arterial hypertension are expected to be slightly higher than in healthy subjects, particularly in patients with a markedly reduced cardiac output.

4.2 Drug–drug Interactions

Different avenues of research were followed to explore the propensity of bosentan to interact with other drugs. Several experiments were conducted *in vitro* and, when considered appropriate, followed up by studies *in vivo*.

Protein binding. Because of bosentan's high extent of plasma protein binding (98%), *in vitro* displacement studies were undertaken. No relevant interactions were found at the level of protein binding with digitoxin, glibenclamide (glyburide), phenytoin, tolbutamide, and warfarin.

Cytochrome P450 inhibition. Bosentan has no inhibitory effect on CYP1A2 and 2D6 and a weak inhibitory effect on CYP2C9, CYP2C19, and CYP3A4. Therefore, bosentan is not expected to increase the plasma concentrations of drugs metabolized by these isoenzymes. Indeed, bosentan did not increase but rather decreased the exposure to several substrates of CYP2C9 and 3A4 (see CYP2C9 and CYP3A4 induction below).

CYP2C9 and CYP3A4 metabolize bosentan and, therefore, plasma concentrations of bosentan may increase as a result of inhibition of these isoenzymes. Ketoconazole, a potent inhibitor of CYP3A4, increased C_{max} and AUC values of bosentan at steady state 1.6- and 1.8-fold, respectively. After the first concomitant administration, ketoconazole increased bosentan trough levels by only 17%. The modest effect of ketoconazole on the pharmacokinetics of bosentan, as compared to drugs solely dependent on CYP3A4 for their metabolism, suggests that the CYP2C9 metabolic pathway limits the magnitude of the change when CYP3A4 is inhibited. Selective inhibitors of CYP2C9 are not available for human use, which precluded the conduct of an *in vivo* interaction study. However, on the basis of *in vitro* experiments, showing that the contribution of CYP3A4 to the metabolism of bosentan is quantitatively more important than that of CYP2C9, inhibition of the latter is not expected to elicit greater effects than seen with ketoconazole.

Cyclosporine A, an inhibitor of CYP3A4, and of drug transporter proteins such as P-glycoprotein, increased the plasma concentrations of bosentan in a multiple-dose interaction study. Upon the first concomitant administration, no full pharmacokinetic profile of bosentan was obtained, only trough levels were determined. These trough levels were increased 31-fold and, subsequently, decreased rapidly. At steady state, exposure to bosentan was 4-fold that expected in the absence

of cyclosporine A. Based on the previously described ketoconazole interaction study, the contribution of inhibition of CYP3A4 appears to be limited to 2-fold. Since bosentan is well absorbed, increased absorption in the presence of cyclosporine A also cannot explain the observed marked increase in initial trough concentrations. It is hypothesized that cyclosporine A inhibits the elimination of bosentan from the systemic circulation via inhibition of drug transporter proteins.

Simvastatin, a substrate of CYP3A4 and losartan, a substrate of CYP2C9, did not exert any influence on the pharmacokinetics of bosentan.

CYP2C9 and CYP3A4 induction. Bosentan is a mild to moderate inducer of CYP2C9, 2C19, and 3A4 as detected in *in vitro* experiments using human hepatocytes. *In vivo*, bosentan increased the plasma concentration ratio of 6β-hydroxycortisol/cortisol 1.6-fold, which is suggestive of CYP3A4 induction. Several *in vivo* interaction studies were conducted to explore the influence of bosentan on the pharmacokinetics of drugs metabolized by the isoenzymes specified.

The effects of bosentan on the pharmacokinetics of cyclosporine A (CYP3A4 substrate), simvastatin and its β-hydroxy acid metabolite (both CYP3A4 substrates), glibenclamide (CYP3A4 substrate), R-warfarin (CYP3A4 substrate), S-warfarin (CYP2C9 substrate) and digoxin (P-glycoprotein substrate with a narrow therapeutic window) were investigated. No interaction studies were conducted with substrates of CYP2C19.

Bosentan reduced the exposure to substrates of CYP2C9 and 3A4 by 30–60%, whereas no effect was demonstrated on digoxin. The possibility of reduced efficacy of CYP2C9 and 3A4 substrates should be considered when co-administered with bosentan. The results of the five drug–drug interaction studies are summarized in Table 1. In these studies, bosentan doses ranged from 125 mg b.i.d. (simvastatin, glibenclamide) to 500 mg b.i.d (cyclosporine A, digoxin, warfarin).

Table 1 Influence of bosentan on the pharmacokinetics of other drugs

Compound	AUC	Possible mechanism
Cyclosporine A	Decreased 49%	CYP3A4 induction
Digoxin	No significant change	—
Glibenclamide (glyburide)	Decreased 40%	CYP3A4 induction
Simvastatin	Decreased 49%	CYP3A4 induction
β-hydroxyacid simvastatin	Decreased 60%	CYP3A4 induction
Warfarin	S-warfarin decreased 29%	CYP2C9 induction
	R-warfarin decreased 38%	CYP3A4 induction

As warfarin is frequently given to patients with pulmonary arterial hypertension, the clinical relevance of a potential interaction between warfarin and bosentan was retrospectively analyzed using data available from Study AC-052-352. Included in this analysis were those 99 patients

(63 bosentan, 36 placebo) who were stable on warfarin therapy before the start of bosentan treatment. The analysis focused on the warfarin dose at the start and end of the study as well as the frequency of warfarin dose modifications due to either a laboratory abnormality (i.e., change in International Normalized Ratio [INR]) or an adverse event (e.g., bleeding). The average dose of warfarin in the bosentan group was 4.3 mg and 4.4 mg at the start and end of the study, respectively. For the placebo group, these values were 5.3 mg and 5.1 mg, respectively. The proportion of patients with a warfarin-dose change was 44.4% in both the bosentan and placebo groups. Therefore, these data indicate the absence of a clinically significant interaction between bosentan and warfarin.

5 CLINICAL TRIAL PROGRAM IN PULMONARY ARTERIAL HYPERTENSION

5.1 Overview of the Program

The clinical development program designed to demonstrate bosentan's efficacy and tolerability in the treatment of pulmonary arterial hypertension consisted of five clinical studies:

AC-052-351:	the first pivotal, randomized, double-blind, placebo-controlled multicenter Phase III study conducted in the US and France
AC-052-352:	the second pivotal, randomized, double-blind, placebo-controlled multicenter Phase III study conducted in North America, Europe, Israel and Australia (BREATHE-1)
AC-052-353:	an open-label extension of AC-052-351 (ongoing)
AC-052-354:	an open-label extension of AC-052-352 (ongoing)
BD14884:	an exploratory Phase I study consisting of an open-label, single-dose i.v. administration followed by randomized, double-blind, placebo-controlled oral treatment

Characteristics and designs of the five trials are provided in Section 5.3.

The development program in pulmonary arterial hypertension is based on two complementary, adequate and well controlled pivotal trials, AC-052-351 and AC-052-352. Both were placebo-controlled studies of oral bosentan taken twice daily. Patients were randomized to bosentan or placebo in a 2:1 manner resulting in a total of 246 patients (214 in AC-052-352 and 32 in AC-052-351). One patient in AC-052-352 did not receive randomized treatment, which resulted in an overall intent-to-treat (ITT) population of 165 patients on bosentan and 80 on placebo (total of 213). Studies AC-052-353 and AC-052-354 are the currently ongoing open-label extensions of the placebo-controlled studies. Of the 245 patients who participated in the two placebo-controlled trials, 29 of 32 patients (91%) in AC-052-351 and 198 of 213 patients (93%) in AC-052-352 (total of 227 patients) continued in the open-label extension trials**.** Data from Study AC-052-353 provide long-term efficacy and safety data on the patients who continued from AC-052-351 (28/31 patients, thus the majority). No data are presented from Study AC-052-354 because data were not available as of the clinical cut-off date.

Study BD14884 was an earlier exploratory Phase I study that had been conducted in Australia by the previous licensee of the compound, F. Hoffmann-La Roche. This study was prematurely stopped after seven patients were enrolled, and only two patients completed the study. Furthermore, as this study used dosages, formulations and routes of administration different than that intended for use, only safety data from this study are presented.

In addition to studies conducted in patients with pulmonary arterial hypertension, bosentan has been studied in a variety of other indications and in healthy subjects. Data from 16 clinical pharmacology studies (N = 487), 7 drug–drug interaction studies (N = 84) and 6 clinical trials (N = 2333) in CHF, systemic hypertension, and SAH have contributed to bosentan's safety evaluation. Data from 1613 of these patients (ENABLE in severe CHF) are still blinded.

5.2 Rationale for the Doses Selected for the Clinical Trials in Pulmonary Arterial Hypertension

Target doses: 125 mg b.i.d. and 250 mg b.i.d.

In previous studies in patients with systemic hypertension or severe CHF, bosentan doses of 100/125 mg b.i.d. were found to be at the top of the dose-response curve for blood pressure reduction. Higher doses of bosentan were associated with an increased incidence of elevated liver aminotransferases. Therefore, the target dose in the first placebo-controlled trial in pulmonary arterial hypertension (AC-052-351) was bosentan 125 mg b.i.d. In order to further explore the dose range in these patients, bosentan 250 mg b.i.d. was evaluated in the second trial (AC-052-352).

Starting dose: 62.5 mg b.i.d.

In the REACH-1 study (NC15462), patients with CHF had a higher risk of worsening heart failure during the first 4 weeks of therapy than those assigned to placebo. This risk appeared to be related to the starting dose of bosentan (125 mg b.i.d. or 250 mg b.i.d. with a target dose of 500 mg b.i.d.) and/or the rapidity of up-titration (weekly increase in dose to 500 mg b.i.d.). Therefore, it was decided that in further trials with bosentan, the starting dose would be 62.5 mg b.i.d., and the up-titration would take place after 4 weeks of treatment.

5.3 Objectives and Study Designs

The main characteristics of the five studies in pulmonary arterial hypertension are shown in Table 2.

Table 2 Main characteristics of the therapeutic studies in patients with pulmonary arterial hypertension

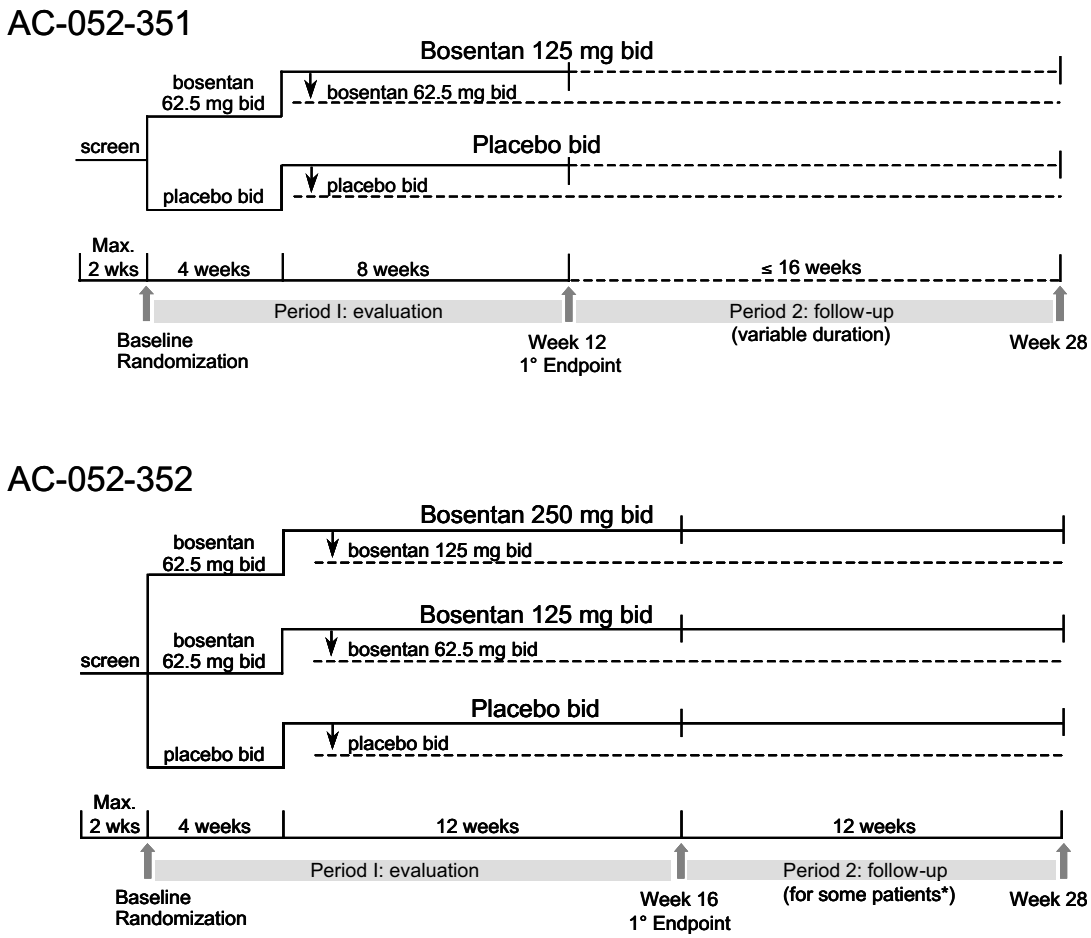
Protocol (Report No.)	Study Objectives	Treatment Duration	Treatment*	No. of Patients Enrolled	
AC-052-351 (B-00.028)	Efficacy and safety	Period I: 12 weeks Period II: variable (Completed)	Bosentan 62.5/125 mg b.i.d. Placebo	Bosentan Placebo	21 11
AC-052-352 (Initial Rept. B-01.015)	Efficacy and safety	Period 1: 16 weeks Period 2: 12 weeks‡ (Completed)	Bosentan 62.5/125 mg b.i.d.† Bosentan 62.5/250 mg b.i.d.† Placebo†	Bosentan Placebo	144 69
AC-052-353 (Interim Rept. B-00.027)	Long-term safety and efficacy	Ongoing	Bosentan 62.5/125 mg b.i.d.	Ex-bosentan Ex-placebo	21 8
BD14884 (B-162292)	Exploratory (safety, efficacy, PK/PD for single i.v. and multiple oral doses)	Part I: 1 day Part II: 8 weeks (Stopped prematurely)	Part I: Bosentan 500 mg i.v. Part II: Bosentan 1000 mg b.i.d. Placebo	Part I: Part II: Bosentan Placebo	7 4 3

* 62.5/125 mg = forced titration after 4 weeks treatment (or after 1 to 4 weeks treatment in Study AC-052-354); 1000 mg = fixed dose.
† half target dose if body weight ≤ 40 kg.
‡ Only patients enrolled by 30 September 2000 were scheduled to complete Period 2.
b.i.d. = twice daily, i.v. = intravenous, PK/PD = pharmacokinetics/pharmacodynamics.

5.3.1 Placebo-controlled Studies

The primary objective of both placebo-controlled studies was to evaluate the effects of bosentan on exercise capacity in patients with pulmonary arterial hypertension. Secondary objectives were to gain safety experience and assess the effects of bosentan on overall clinical status (e.g., WHO functional class and time to clinical worsening) and on hemodynamics (in Study AC-052-351 only). Patients were randomized to double-blind treatment after a screening period of ≤ 2 weeks (Figure 10). In Study AC-052-351, randomization was 2:1 to bosentan 125 mg b.i.d or placebo, and in AC-052-352, patients were equally randomized to bosentan 125 mg b.i.d., bosentan 250 mg b.i.d. or placebo (resulting in a bosentan to placebo ratio of 2:1). Patients in both studies were started on bosentan 62.5 mg b.i.d. or placebo and up-titrated to the target dose after 4 weeks (half the target dose if ≤ 40 kg in AC-052-352). The dose could be down-titrated by half at any time after the first week at the target dose for reasons of tolerability or safety. In AC-052-352, patients who prematurely withdrew were offered 3 to 7 days of low-dose treatment (62.5 mg b.i.d. or placebo) to wean from the higher dose.

Figure 10 Study designs for pivotal studies in pulmonary arterial hypertension



* The 48 patients who entered the study up to 30 September 2000 were scheduled to participate.

The primary evaluation period (Period 1) was 12 weeks in Study AC-052-351 and 16 weeks in AC-052-352. After completion of Period 1, patients in Study AC-052-351 continued treatment in Period 2 for an additional 16 weeks or until the last enrolled patient not prematurely discontinued completed Period 1, upon which the study was ended (variable participation in Period 2). In Study AC-052-352, patients who were entered up to 30 September 2000 were scheduled to participate in Period 2 (n = 48), which consisted of up to an additional 12 weeks of treatment until the last entered patient not prematurely discontinued completed Period 1 and the study was ended.

Patients were periodically assessed for exercise capacity (6-minute walk test), dyspnea on exercise (Borg dyspnea index), WHO functional class, clinical deterioration (worsening), clinical laboratory tests, 12-lead electrocardiogram (ECG), and vital signs. Adverse events (AEs) and

changes in concomitant treatments were continually monitored. Patients in Study AC-052-351 also had central hemodynamics assessed via a Swan-Gantz catheter after 12 weeks of treatment; hemodynamics were assessed after the 6-minute walk test. The primary efficacy endpoint in both studies was the change from baseline to the end of Period 1 in walk distance as assessed by the 6-minute walk test.

5.3.2 Open-label Extension Studies

The main objective of the open-label extension study (AC-052-353) was to collect long-term safety data on bosentan in patients with pulmonary arterial hypertension. Patients who completed the study or who discontinued during Period 2 for one of the predefined reasons (AE requiring withdrawal, clinical deterioration as judged by the investigator, lack of improvement in walk distance) and who completed the premature-discontinuation/end-of-study assessments were eligible to immediately enter the extension study. Patients who were discontinued during Period 1 of the placebo-controlled study were not eligible.

Patients were started on bosentan 62.5 mg b.i.d. and were to be up-titrated to 125 mg b.i.d. at Visit 1 (typically at Week 4) unless the patient did not tolerate the higher dose. This included patients who had been treated with bosentan in the previous study, since most patients were still blinded as to previous treatment. Up-titration may have occurred as early as Week 1 if judged by the investigator to be in the patient's best interest (i.e., because of early worsening of the PHT clinical condition). The dose could have been down-titrated by half at any time for reasons of intolerance or safety, and patients were to continue treatment indefinitely.

Visits were scheduled at baseline and after 4 and 12 weeks of treatment, and every 6 months thereafter. Patients were assessed for exercise capacity (6-minute walk test) at visit 1, and at all visits, patients were assessed for WHO functional class, clinical laboratory tests, and vital signs and AEs and concomitant medications were recorded.

5.3.3 Exploratory Study (BD14884)

Study BD14884 was an exploratory study conducted at a single center in Australia that consisted of two parts, an open-label i.v. administration of bosentan on Day 1 (Part I) followed by an 8-week randomized, double-blind administration of oral bosentan or placebo (Part II). With the exception of diuretics, background cardiovascular medications including calcium channel blockers were removed 24 hours before right heart catheterization prior to the i.v. infusion in Part I of the study. High dosages of bosentan were used in this study. The 500 mg infusion (administered as 50 mg/5 min, 150 mg/10 min followed by 300 mg/15 min at 2-hour intervals) on Day 1 was followed by oral bosentan 1000 mg b.i.d. or placebo. The primary objective was to assess safety, but additional objectives were to assess the efficacy, pharmacokinetics and pharmacodynamics of single i.v. and multiple oral doses of bosentan. The study was prematurely stopped for reasons of patient safety after only 7 of the planned 30 patients had enrolled. All seven patients participated in Part I (i.v. infusion of open-label bosentan) on Day 1 and were randomized to oral treatment, 4 to bosentan and 3 to placebo, starting on Day 2. The study was stopped when two of the patients on placebo died on Day 3, and only one patient in each

treatment group completed the study. Because the study was prematurely stopped, few data were collected. Safety data from this study have been summarized in Appendix 1.

5.4 Patient Population

The target population for the clinical program included ambulatory adult outpatients with symptomatic severe pulmonary arterial hypertension due to primary pulmonary hypertension or secondary to systemic connective tissue disease (e.g., scleroderma or systemic lupus erythematosus). Patients whose disease was due to other causes (e.g., congenital heart disease, HIV, cirrhosis, thromboembolic disease, chronic obstructive pulmonary disease) and those with SSc/PHT who had moderate to severe interstitial fibrosis were not included. Patients were required to be ≥ 12 years of age (> 18 years in Study AC-052-351) and in WHO functional class III or IV with a baseline mean pulmonary arterial pressure (PAP) > 25 mmHg, a pulmonary vascular resistance (PVR) >3 mmHg/l/min (240 dyn·sec/cm^5), and a pulmonary capillary wedge pressure (PCWP) < 15 mmHg. At randomization, patients were required to have a mean baseline walk test between 150 m and 450 m (500 m in AC-052-351), inclusive (mean of two assessments with ≤ 15% difference). Patients were excluded if they had a systolic blood pressure < 85 mmHg, ALT or aspartate aminotransferase (AST) concentrations > 3 × ULN, hemoglobin or hematocrit > 30% below the lower limit of normal (LLN), treatments for pulmonary arterial hypertension modified within 1 month of screening (excluding anticoagulants) or had received epoprostenol within 3 months of screening.

The demographic and disease characteristics of pulmonary arterial hypertension patients in the two pivotal trials are summarized in Table 3. In both placebo-controlled studies, treatment groups were well matched.

Table 3 Demographic and baseline disease characteristics of studied patients

	AC-052-352		AC-052-351	
	Placebo (n = 69)	Bosentan (n = 144)	Placebo (n = 11)	Bosentan (n = 21)
Demographics				
Gender (% M/F)	22 / 78	21 / 79	0 / 100	19 / 81
Age (years)				
Mean ± SD	47 ± 16	49 ± 16	47 ± 14	52 ± 12
Min, Max	12, 80	13, 80	25, 67	33, 73
Weight (kg)				
Mean ± SD	74 ± 18	71 ± 20	87 ± 18	86 ± 23
Min, Max	33, 123	36, 137	55, 113	57, 147
Race (%W/B/O)	86 / 1 / 13	77 / 8 / 15	82 / 18 / 0	76 / 14 / 10
Etiology of PAH [n (%)]				
PPH	48 (69.6)	102 (70.8)	10 (90.9)	17 (81.0)
SSc/PHT	14 (20.3)	33 (22.9)	1 (9.1)	4 (19.0)
Other	7 (10.1)	9 (6.3)	0	0
Time from diagnosis of PAH (days)*				
Mean ± SD	843 ± 1442	896 ± 1062	1091 ± 1032	634 ± 528
Median	381	453	909	455
Min, Max	7, 9923	5, 5259	30, 2717	29, 1666
Baseline assessments (mean ± SD)				
WHO class (% III/IV)	94 / 6	90 / 10	100 / 0	100 / 0
6-min walk test (m)	344.3 ± 76.4	329.6 ± 74.1	355.5 ± 81.8	360.5 ± 86.1
Mean PAP (mmHg)	53.4 ± 16.6	54.7 ± 15.8	56.5 ± 10.3	52.7 ± 13.8
PVR (dyn·sec/cm^5)	880 ± 540†	1014 ± 678†	989 ± 435†	866 ± 435
Cardiac index (l/min/m^2)	2.43 ± 0.69	2.35 ± 0.82	2.39 ± 1.03	2.40 ± 0.74

* Reported number of days from diagnosis of pulmonary hypertension to randomization.

† PVR could not be calculated for some patients who did not have a PCWP measurement (n = 65 and n = 135 for placebo and bosentan groups in AC-052-352, and n = 19 in the bosentan group, in AC-052-351).

B = Black, F = female, M = male, O = other, PAH = pulmonary arterial hypertension, PAP = pulmonary arterial pressure, PCWP = pulmonary capillary wedge pressure, PPH = primary pulmonary hypertension, PVR = pulmonary vascular resistance, SD = standard deviation, SSc/PHT = pulmonary hypertension due to scleroderma, W = white.

The main previous and concomitant treatments for pulmonary arterial hypertension (taken by more than 10% of patients) in both placebo-controlled studies are shown in Table 4. The majority of patients were taking an anticoagulant (primarily warfarin) and at least one diuretic (usually furosemide and/or spironolactone). In Study AC-052-351, calcium channel blockers were almost exclusively diltiazem or amlodipine whereas in Study AC-052-352, most patients were taking nifedipine or diltiazem.

Table 4 Main previous and concomitant treatments for pulmonary arterial hypertension in Studies AC-052-351 and AC-052-352, safety populations

	AC-052-352		AC-052-351	
	Placebo (n = 69)	All bosentan (n = 144)	Placebo (n = 11)	Bosentan (n = 21)
Anticoagulants	49 (71.0)	96 (66.7)	9 (81.8)	15 (71.4)
Diuretics	35 (50.7)	77 (53.5)	10 (90.9)	18 (85.7)
Calcium channel blockers	36 (52.2)	64 (44.4)	6 (54.5)	11 (52.4)
Cardiac glycosides	13 (18.8)	28 (19.4)	1 (9.1)	3 (14.3)
Oxygen	24 (34.8)	41 (28.5)	1 (9.1)	6 (28.6)

Pts = patients

5.5 Disposition of Patients

In AC-052-351, three patients were withdrawn due to worsening of pulmonary arterial hypertension (two during Period 1 and one during Period 2). All three patients had been on placebo and were started on epoprostenol. No deaths occurred during the study, and all the remaining patients were enrolled in the extension study (AC-052-353).

In AC-052-352, a total of 30 patients (19 on bosentan and 11 on placebo) were prematurely discontinued from the study (Table 5). However, 9 (6.3%) patients on bosentan and 3 (4.3%) on placebo were discontinued from Period 2 due to administrative reasons (in order to enroll in the open-label study). Seven of these patients had not been scheduled to enter Period 2, but had continued treatment in the study because the open-label extension was not yet available at their site. When participation in the open-label extension was possible, these patients were withdrawn from Study AC-052-352.

Table 5 Premature discontinuations in Study AC-052-352, safety population

(T02c / 04MAY01)

Reason for premature discontinuation	Bosentan 125 mg N=74 No.	%	Bosentan 250 mg N=70 No.	%	All Bosentan N=144 No.	%	Placebo N=69 No.	%
Total pts with at least one reason	10	13.5%	9	12.9%	19	13.2%	11	15.9%
ADMINISTRATIVE/OTHER	6	8.1%	3	4.3%	9	6.3%	3	4.3%
WORSENING OF PATIENT CONDITION	3	4.1%	2	2.9%	5	3.5%	5	7.2%
INCREASED LIVER ENZYMES	-		3	4.3%	3	2.1%	-	
DEATH	1	1.4%	-		1	0.7%	2	2.9%
AE/INTERCURRENT ILLNESS	-		1	1.4%	1	0.7%	-	
LACK OF CLINICAL/WALK TEST IMPROVEMENT	-		-		-		1	1.4%

AE = adverse event, Pts = patients

As of the cut-off date (31 March 2001 or the 12-month assessment, if later), only one patient has been withdrawn from the extension study (AC-052-353). Patient 10503 was withdrawn on Day 105 because of worsening pulmonary arterial hypertension and put on epoprostenol. The investigator attributed the worsening to a lack of compliance with bosentan treatment. No patients have died in this study.

5.6 Exposure to Study Medication

Because of the designs of the placebo-controlled studies, patients received treatment for different durations. In Study AC-052-351, all patients should have received randomized treatment for 12 weeks (Period 1) and continued treatment in Period 2 until the trial was completed. This resulted in a variable treatment duration. In AC-052-352, all 213 patients should have received randomized treatment for 16 weeks (Period 1), and the 48 patients who entered the study before 30 September 2000 were scheduled to receive treatment for up to an additional 12 weeks (Period 2). However, because the open-label extension was not yet available at some sites, several patients who were not scheduled to continue treatment in Period 2 did so until the open-label study was available.

In both open-label extension studies, patients are being treated on an ongoing basis.

A summary of exposure to treatment in patients with pulmonary arterial hypertension is provided in Table 6, Figure 11, and Figure 12.

Table 6 Exposure to bosentan in patients with pulmonary arterial hypertension

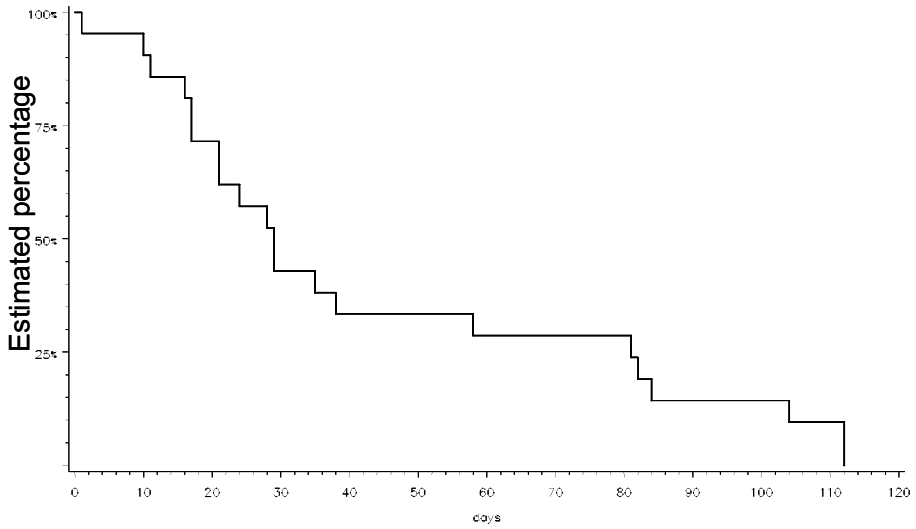
	AC-052-351	AC-052-352		AC-052-353*
	125 mg b.i.d. (n = 21)	125 mg b.i.d. (n = 74)	250 mg b.i.d. (n = 70)	125 mg b.i.d. (n = 29)
Duration of treatment (days)				
Mean	132	129.4	128.7	365
Standard deviation	36	35.8	32.4	51
Median	119	118	118	374
Min, Max	83, 197	24, 207	64, 204	105, 411
Number (%) of patients who remained at target dose†	21 (100)	56 (75.7)	55 (78.6)	27 (93.1)†

* As of clinical cut-off on 31 March 2001 or at the 12-month visit, if later.

† For four patients in Study AC-052-353, the dose was increased to 250 mg b.i.d. after 348 to 379 days of treatment.

Patients on bosentan in Study AC-052-351 were treated for a median of about 17 weeks (range of 12 to 28 weeks as expected per protocol). All bosentan patients completed Period 1, received treatment in Period 2 as described in Figure 11, and continued treatment in the open-label extension (AC-052-353). As of the clinical cut-off date, patients in AC-052-353 have been treated for a median of about 53 weeks (range of 15 to 59 weeks). Patients who were on bosentan in the placebo-controlled study (AC-052-351) have received bosentan for a total combined mean ± standard deviation (SD) duration of 505 ± 41 days (median 488 days, range 443 to 594 days), or for about 1.4 years.

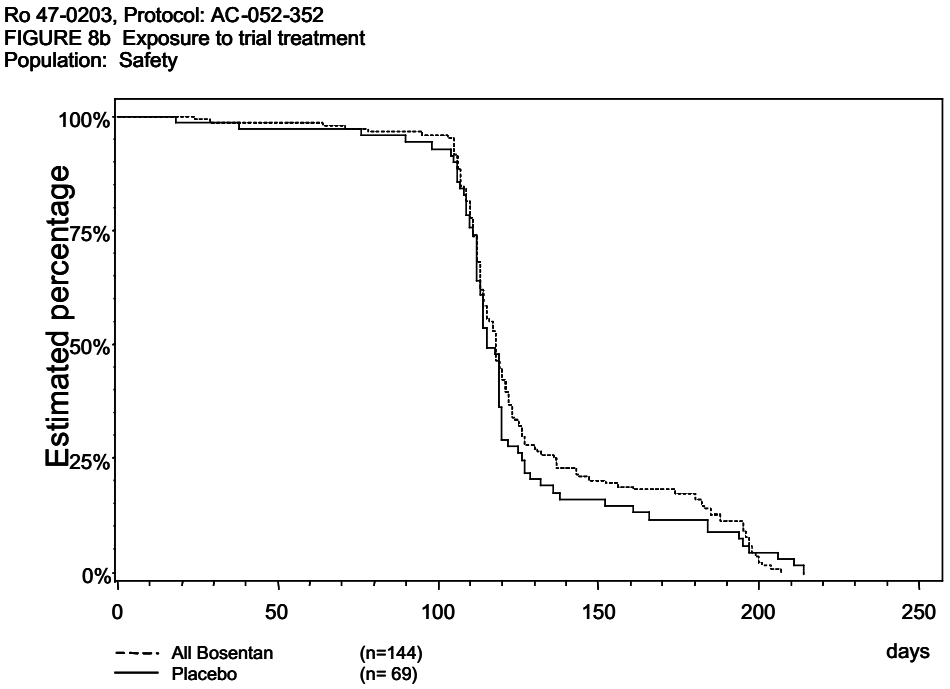
Figure 11 Exposure to bosentan in Study AC-052-351 during Period 2



Note: All patients randomized to bosentan were exposed for 12 weeks during Period 1 in addition to the time in Period 2.

Patients in both bosentan dosage groups in Study AC-052-352 were treated for a median of about 17 weeks (ranges of 3 to 30 weeks in the 125 mg b.i.d. group and 9 to 29 weeks in the 250 mg b.i.d. group). Exposure was similar between the dosage groups and equally similar to that in the placebo group (Figure 12).

Figure 12 Exposure in AC-052-352 during Periods 1 and 2



6 EFFICACY OF BOSENTAN IN PULMONARY ARTERIAL HYPERTENSION

In the two pivotal, placebo-controlled studies (AC-052-351 and AC-052-352), all efficacy parameters were analyzed in the ITT populations. The ITT populations included all randomized patients who received any study treatment and who had a valid assessment of the primary endpoint after applying the predefined substitution rules for missing data. The main per protocol efficacy objective in both trials was to compare all bosentan-treated patients to placebo-treated patients.

In both studies, the primary endpoint was the change from baseline to the end of Period 1 (Week 12 in AC-051 and Week 16 in AC-052-352) in walk distance as assessed by the 6-minute walk test. The main per protocol analyses were the evaluation of the primary endpoint in bosentan- versus placebo-treated patients using the Student's t-test in Study AC-052-351 and the

Mann-Whitney U-test in Study AC-052-352. Per protocol, the effect of each dose versus placebo in Study AC-052-352 was analyzed descriptively.

The main secondary efficacy parameters in both studies were the time to clinical worsening (to be analyzed using Kaplan-Meier techniques, per protocol) and the changes in Borg dyspnea index and WHO functional class (analyzed descriptively, per protocol, with mean or proportion and 95% confidence limits [CL]). Hemodynamic effects (change from baseline to Week 12) were additional secondary endpoints in Study AC-052-351, and were also analyzed descriptively, with mean and median change and 95% CL.

In the extension study (AC-052-353), the 6-minute walk test was performed at the Week 4 assessment, and WHO functional class was assessed at each clinic visit. Long-term efficacy data from Study AC-052-353 were exploratory and analyzed in the safety population.

6.1 Exercise Capacity (Primary Endpoint)

In both pivotal trials, patients on bosentan achieved a significantly greater and clinically meaningful increase in walk distance than did those on placebo (Table 7 and Table 8). In the first study (AC-052-351), a mean placebo-corrected treatment effect of 75.9 meters (95% CL of 12.5 and 139.2 meters) in favor of bosentan was observed ($p = 0.0205$). The second and larger study (AC-052-352) confirmed the findings in the initial study. Treatment with bosentan resulted in a mean placebo-corrected treatment effect of 44.2 meters (95% CL of 21.4 and 67.0 meters), and the greater increase in walk distance with bosentan was significant at $p = 0.0002$. In both studies, missing values were substituted with zero meter value when patients were prematurely withdrawn for reasons of death or worsening of pulmonary arterial hypertension without a premature withdrawal assessment, as per protocol.

Table 7 Walk test: Change from baseline to Week 12 in Study AC-052-351, ITT population

(Table T15 / 18AUG00)

	Bosentan N=21	Placebo N=11
Baseline (m)		
Mean	360.5	355.5
SD	86.1	81.8
Std err	18.8	24.7
Median	380.0	405.5
Min , Max	218 , 483	218.5 , 437
Week 12* (m)		
Mean	430.5	349.6
SD	66.4	147.1
Std err	14.5	44.4
Median	431.0	399.0
Min , Max	294 , 535	0 , 497
Change (m)		
Mean	70.1	-5.8
SD	56.2	120.5
Std err	12.3	36.3
95% CL of mean	44.5 , 95.6	-86.8 , 75.2
Median	51.0	-6.0
Min , Max	-24.5 , 196	-267.5 , 224.5
Placebo-corrected Treatment Effect (m)		
Mean	75.9	
Std err	31.0	
95% CL of mean	12.5 , 139.2	
Median	59.0	
95% CL of median	13.2 , 130.3	
p-value t-test	0.0205	
p-value Mann-Whitney U-test	0.0190	

(*) Two patients (1 bosentan, 1 placebo) had no week 12 visit and had the last valid value carried forward. Only 1 placebo patient withdrew from the study without a valid end of study assessment; a value of 0 m was used for the week 12 assessment.
SD = standard deviation: Std err = standard error

Table 8 Walk test: Change from baseline to Week 16 in Study AC-052-352, ITT population

(Table T09 / 09MAY01)

Walk test (m)	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
Use of supplemental oxygen during screening/randomization walk tests				
n	74	70	144	69
Yes	11 14.9%	12 17.1%	23 16.0%	16 23.2%
No	63 85.1%	58 82.9%	121 84.0%	53 76.8%
Baseline				
n	74	70	144	69
Mean	326.3	333.0	329.6	344.3
Standard deviation	73.2	75.4	74.1	76.4
95% CL of mean	309.3 , 343.2	315.0 , 351.0	317.4 , 341.8	326.0 , 362.7
Median	333.0	338.8	337.3	359.0
95% CL of median	306.5 , 357.5	316.0 , 369.0	320.0 , 357.0	344.0 , 382.5
Min , Max	159.0 , 464.5	173.5 , 440.0	159.0 , 464.5	150.0 , 448.5
Week 16				
n	74	70	144	69
Mean	353.1	379.5	365.9	336.5
Standard deviation	115.0	101.2	109.0	129.2
95% CL of mean	326.4 , 379.7	355.3 , 403.6	348.0 , 383.9	305.4 , 367.5
Median	376.5	384.5	379.5	355.0
95% CL of median	338.0 , 396.0	363.0 , 417.0	363.0 , 396.0	333.0 , 378.0
Min , Max	0.0 , 602.0	57.0 , 555.0	0.0 , 602.0	0.0 , 585.0
Change from baseline				
n	74	70	144	69
Mean	26.8	46.5	36.4	-7.8
Standard deviation	75.3	61.7	69.5	96.1
95% CL of mean	9.3 , 44.2	31.7 , 61.2	24.9 , 47.8	-30.9 , 15.2
Median	32.8	49.8	34.5	9.0
95% CL of median	19.5 , 40.0	19.5 , 66.0	26.0 , 48.5	-18.0 , 26.0
Min , Max	-205.0 , 214.0	-131.0 , 257.5	-205.0 , 257.5	-383.0 , 227.5
PLACEBO-CORRECTED TREATMENT EFFECT (m)				
Mean	34.6	54.3	44.2	
95% CL of mean	6.2 , 63.1	27.3 , 81.4	21.4 , 67.0	
Median	28.2	45.0	36.7	
95% CL of median	7.5 , 51.5	23.1 , 67.1	17.9 , 55.9	
p-value Mann-Whitney U-test			0.0002	

CL=confidence limits.

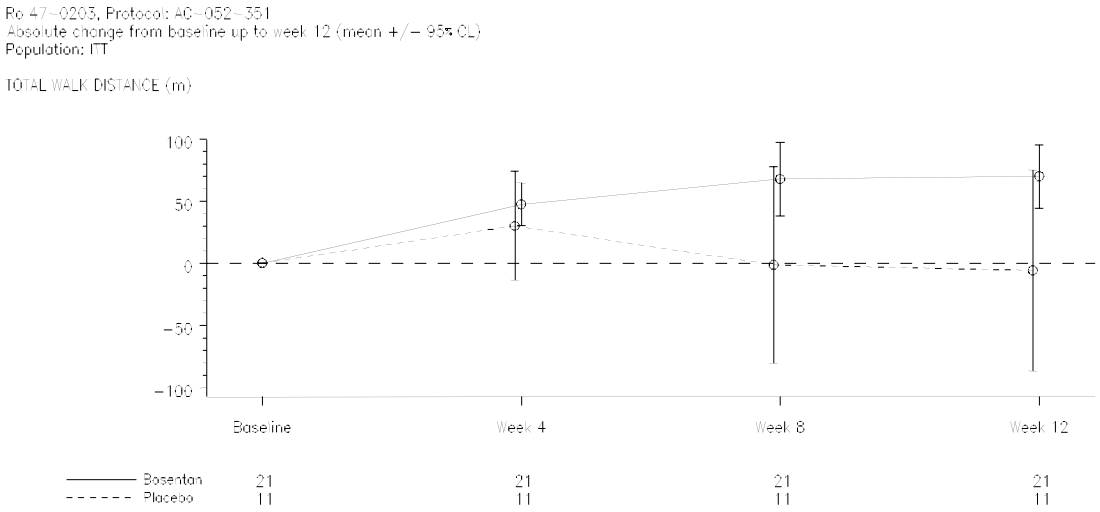
The robustness of results was checked in both trials with similar findings in all analyses. In Study AC-052-351, results were also analyzed using a non-parametric test (Mann-Whitney U-test) because of the unequal distribution due to the application of the substitution rules and an outlier patient. In this analysis, bosentan also demonstrated a significant treatment effect ($p = 0.019$). A further analysis using altered and more conservative substitution rules (missing value replaced by last value carried forward) also showed a significant difference between the treatment groups ($p = 0.041$, Student's t-test). In this trial, the standard population (per protocol) was identical to the ITT population.

In Study AC-052-352, using the altered substitution rules (missing value replaced by last value carried forward) also produced results similar to those of the primary analysis ($p = 0.0002$, Mann-Whitney U-test). Because the number of patients in this study was greater than that planned per protocol, a further analysis was performed in which the first 150 patients enrolled were included. Results from this analysis confirmed the significant improvement in exercise capacity with bosentan ($p = 0.0063$, Mann-Whitney U-test). Analysis of the primary endpoint in the standard (per protocol) population resulted in a similar mean treatment effect to that seen in the ITT population ($p = 0.0011$, Mann-Whitney U-test).

6.1.1 Effect Over Time

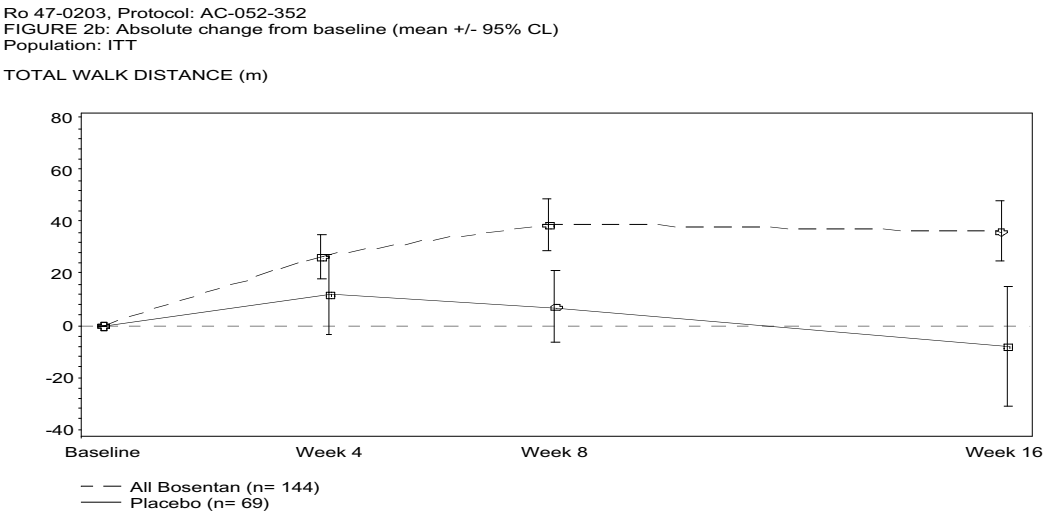
The increase in walk distance observed during Period 1 with bosentan was apparent as early as Week 4 with the 62.5 mg b.i.d. dose in both studies (Figure 13 and Figure 14). The significant increases from baseline at Week 4 were followed by further increases up to Week 8 (with the target dose), and were maintained at the Week-12 (AC-052-351) and Week-16 (AC-052-352) assessments. In contrast, the initial non-significant mean increase from baseline with placebo was lost with a subsequent continual decrease over time in both studies.

Figure 13 Walk test: Change from baseline over time during Period 1 (12 weeks) in Study AC-052-351, ITT population



Note: All bosentan patients received 62.5 mg b.i.d. during the first 4 weeks of the study and then were up-titrated to the target dose (125 mg b.i.d.). Replacement rules used for analysis of the primary parameter were applied in this analysis.

Figure 14 Walk test: Change from baseline over time during Period 1 (16 weeks) in Study AC-052-352, ITT population

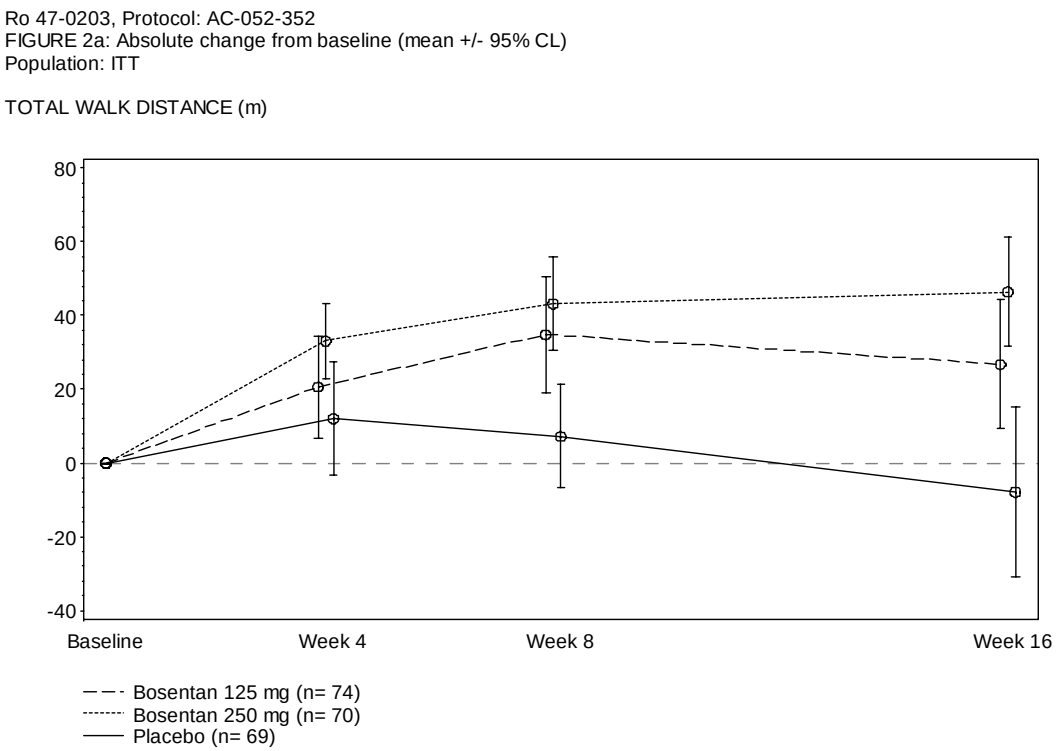


Note: All bosentan patients received 62.5 mg b.i.d. during the first 4 weeks of the study and then were up-titrated to the target dose (125 mg b.i.d. or 250 mg b.i.d.). Replacement rules used for analysis of the primary parameter were applied in this analysis.

6.1.2 Dose Response

When bosentan patients in Study AC-052-352 were categorized by dose (Table 8), significant mean and median treatment effects were obtained with each dose of bosentan compared with placebo ($p = 0.0107$ and $p = 0.0001$ for the 125 and 250 mg b.i.d. doses, respectively, using the Mann-Whitney U-test in exploratory analyses). There was an apparently greater effect with bosentan 250 mg b.i.d. than with 125 mg b.i.d. (means of 54.3 meters and 34.6 meters, respectively), but the difference between the two dose groups was not significant (*ad hoc* analysis) and was already observed at the Week 4 assessment (Figure 15), when patients in both dose groups were receiving the same starting dose (62.5 mg b.i.d.).

Figure 15 Walk test: Change from baseline over time during Period 1 by dose in Study AC-052-352, ITT population

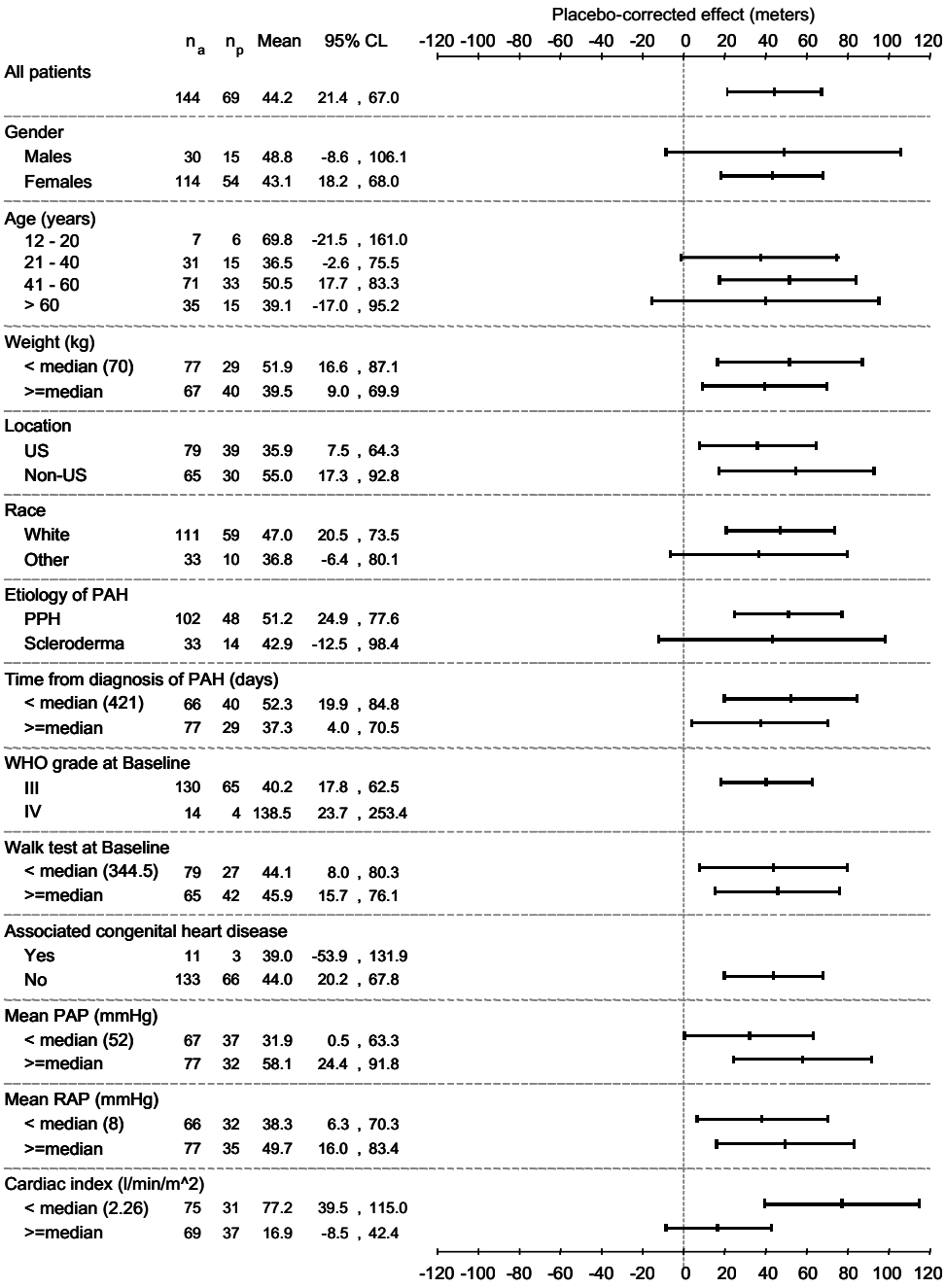


Note: All bosentan patients received 62.5 mg b.i.d. during the first 4 weeks of the study and then were up-titrated to the target dose (125 mg b.i.d. or 250 mg b.i.d.). Replacement rules used for analysis of the primary parameter were applied in this analysis.

6.1.3 Results in Subpopulations

The effect of bosentan treatment compared with placebo as assessed by the change in walk distance was analyzed in subpopulations of patients defined by demographics, disease characteristics and baseline parameters (walk test and hemodynamics) in Study AC-052-352. Mean treatment effects consistently showed improvement with bosentan in all subpopulations assessed, and the increase in walk distance was significantly greater than with placebo in most subpopulations (Figure 16). There were too few patients in Study AC-052-351 to determine any effects in these subpopulations. However, when the two studies were combined, results were similar to those for Study AC-052-352.

Figure 16 **Walk test: Placebo-corrected change from baseline to Week 16 in subpopulations of Study AC-052-352, ITT population**



6.2 Borg Dyspnea Index

Dyspnea during exercise, graded by the patient immediately following the walk test (see Appendix 2), was decreased in all bosentan groups in both placebo-controlled studies and was increased in patients on placebo (Table 9 and Appendix 3). The mean placebo-corrected treatment effect approached statistical significance in both trials. These results were obtained after substituting last value carry forward (or the worst score in cases of death or discontinuation due to worsening pulmonary arterial hypertension without a withdrawal assessment) for missing values, as per protocol. The improvements in dyspnea among bosentan-treated patients are of special clinical importance as they were achieved with a greater walk distance.

Table 9 Borg dyspnea index: Change from baseline to end of Period 1, ITT population

	AC-052-352		AC-052-351	
	125/250 mg b.i.d. (n = 144)	Placebo (n = 69)	125 mg b.i.d. (n = 21)	Placebo (n = 11)
Baseline (m)				
Mean ± SD	3.6 ± 2.0	3.8 ± 2.0	4.38 ± 1.80	4.18 ± 1.94
Median	3.0	3.0	4.00	4.00
Min, Max	0, 10	0, 10	2, 9	2, 8
End of Period 1* (m)				
Mean ± SD	3.3 ± 2.5	4.2 ± 2.4	4.19 ± 2.42	5.55 ± 3.21
Median	3.0	4.0	3.00	4.00
Min, Max	0, 10	0, 10	1, 10	2, 10
Change (m)				
Mean ± SD	−0.3 ± 2.0	0.3 ± 2.0	−0.19 ± 1.66	1.36 ± 2.69
95% CL of mean	−0.6, 0.0	−0.2, 0.8	−0.95, 0.57	−0.45, 3.17
Median	0.0	0.0	0.00	0.00
95% CL of median	0.0, 0.0	0.0, 0.0	−1.00, 1.00	0.00, 5.00
Min, Max	−6.5, 6.0	−3.0, 7.0	−4.0, 4.0	−2.0, 7.0
Placebo-corrected Treatment Effect (m)				
Mean ± SEM	−0.6		−1.55	
95% CL of mean	−1.2, −0.1		−3.12, 0.01	
Median	−0.3		−1.06	
95% CL of median	−1.0, 0.1		−2.94, 0.46	

* Period 1 was 16 weeks in Study AC-052-352 and 12 weeks in Study AC-052-351.

CL = confidence limits, SD = standard deviation, SEM = standard error of the mean.

6.3 Clinical Worsening

Clinical worsening was defined per protocol as the combined endpoint of death, lung transplantation, hospitalization or discontinuation due to worsening pulmonary arterial hypertension, receipt of epoprostenol therapy, or septostomy. However, there were no cases of lung transplantation or septostomy in either study. In both studies, the shortest time from randomization to clinical worsening was to be assessed over the duration of the study (i.e., Kaplan-Meier estimates based on patients on treatment at time points up to 28 weeks).

In Study AC-052-351, only three patients experienced clinical worsening, and the analysis of time to clinical worsening was not performed. All three patients were in the placebo group and were prematurely discontinued from study treatment. No cases of clinical worsening were observed among patients receiving bosentan (p = 0.033 compared with placebo, Fisher's exact test).

In Study AC-052-352, the time to clinical worsening was analyzed using survival analysis techniques (Kaplan-Meier, Figure 17 and Appendix 4). The risk of clinical worsening was significantly less in patients on bosentan than in those on placebo (p = 0.0015). This difference was already significant at Week 16 (p = 0.0038 using the Logrank test in an exploratory analysis with data censored at the end of Period 1, Appendix 5). Compared with placebo, there was significantly less risk of clinical worsening with each of the bosentan doses (p = 0.0133 for 125 mg b.i.d. and p = 0.0122 for 250 mg b.i.d.), and there was no difference between the two dose groups at any time point (Figure 18). As with the combined bosentan patients, the treatment effect was apparent with each dose by the end of Period 1 (p = 0.0298 with 125 mg b.i.d. and p = 0.0197 with 250 mg b.i.d.).

Figure 17 **Time from randomization to clinical worsening up to Week 28 in Study AC-052-352, ITT population**

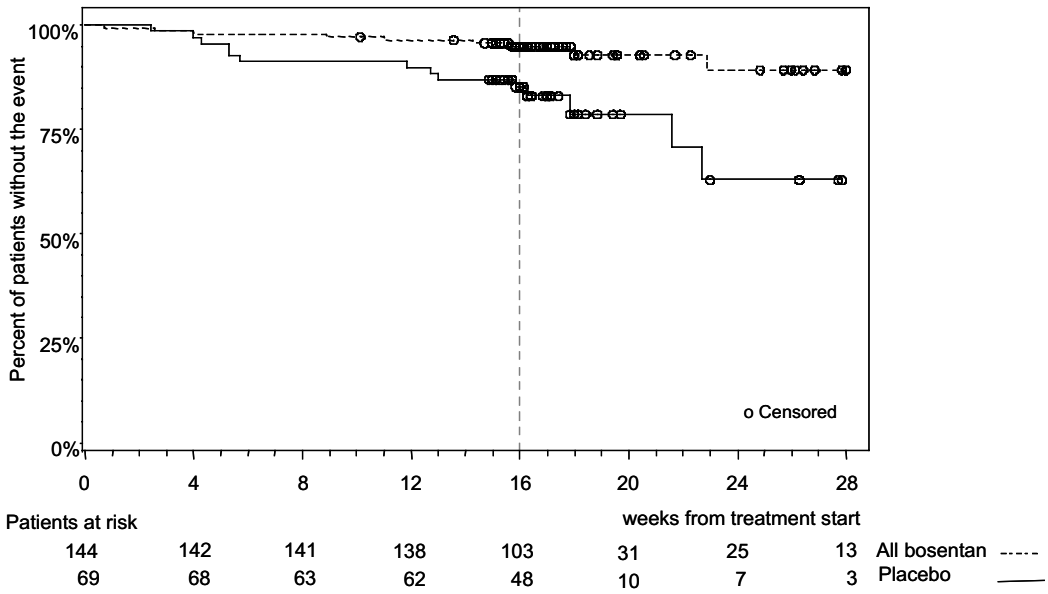
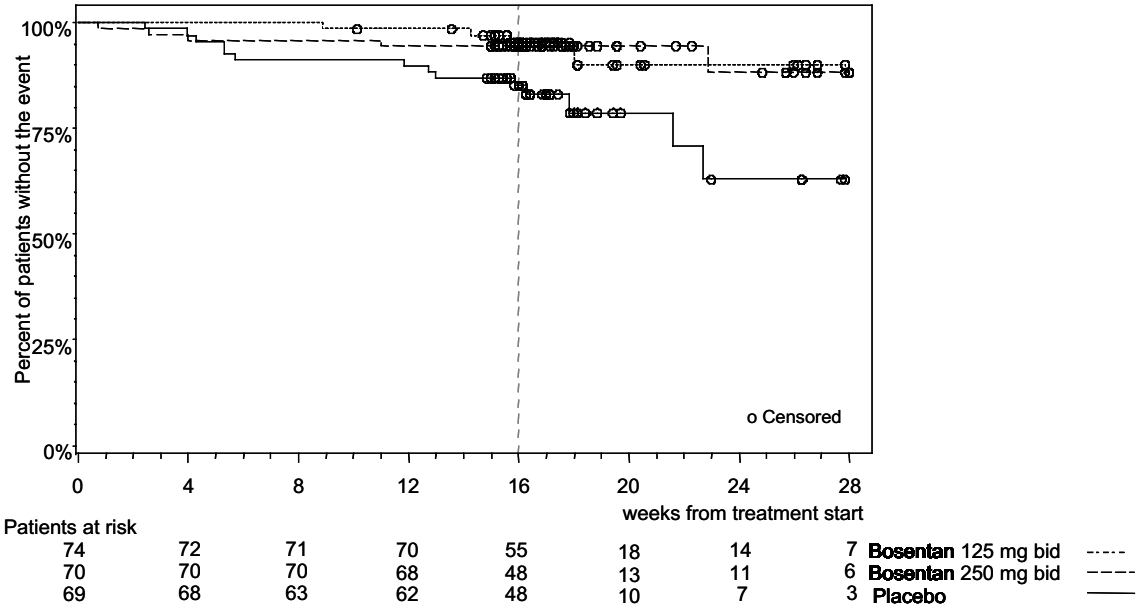


Figure 18 **Time from randomization to clinical worsening by dose in Study AC-052-352, ITT population**



The incidences of clinical worsening during the two trials and the reasons for clinical worsening demonstrate that not only did more patients on placebo experience clinical worsening, but that each component of the composite endpoint occurred in a greater proportion of patients on placebo than in those on bosentan (Table 10 and Appendix 6).

Table 10 Incidence of clinical worsening, ITT population

	AC-052-352		AC-052-351	
	125/250 mg b.i.d. (n = 144)	Placebo (n = 69)	125 mg b.i.d. (n = 21)	Placebo (n = 11)
Patients with clinical worsening [n (%)]	9 (6.3)	14 (20.3)	0	3 (27.3)
Death	1 (0.7)	2 (2.9)	—	—
Hospitalization for PAH	6 (4.2)	9 (13.0)	—	3 (27.3)
Worsening of patient condition*	5 (3.5)	6 (8.7)	—	3 (27.3)
Receipt of epoprostenol	4 (2.8)	3 (4.3)	—	3 (27.3)

Note: Patients may have had more than one reason for clinical worsening.

* Patients who were discontinued due to a worsening of their PAH condition.

PAH = pulmonary arterial hypertension.

6.4 WHO Functional Class

Greater proportions of patients on bosentan than on placebo in both studies showed an improvement in WHO functional class at the end of Period 1, but in neither study did the difference reach statistical significance in the planned exploratory analysis (Table 11 and Appendix 7). These results were obtained after substituting last value carry forward (or the worst class in cases of death or discontinuation due to worsening pulmonary arterial hypertension without a withdrawal assessment) for missing values.

In Study AC-052-352, the distribution of the change from baseline in WHO class was significantly better among bosentan-treated patients than among placebo-treated patients ($p = 0.042$ using the Mann-Whitney U-test in an *ad hoc* exploratory analysis, with median treatment effect −0.5, 95% CL −1.0 and −0.0). In Study AC-052-351, the distribution of change in WHO class was also significantly better among bosentan treated patients ($p = 0.019$ using the Mann-Whitney U-test in an *ad hoc* analysis). All of the patients in AC-052-351 entered the study in WHO class III, and after 12 weeks of treatment, 9 (42.9%) patients on bosentan had improved to class II and none had deteriorated to class IV. In contrast, only 1 (9.0%) of the placebo patients was in class II at the end of Period 1, 2 (18.2%) had deteriorated to class IV, and the majority (72.7%) remained in class III.

Table 11 WHO functional class: Number of patients improved at the end of Period 1, ITT population

	AC-052-352		AC-052-351	
	125/250 mg b.i.d. (n = 144)	Placebo (n = 69)	125 mg b.i.d. (n = 21)	Placebo (n = 11)
n	144	69	21	11
Improved [n (%)]	61 (42.2)	21 (30.4)	9 (42.9)	1 (9.1)
95% confidence limits	34.2, 50.9	19.9, 42.7	21.8, 66.0	0.2, 41.3
Treatment effect*				
Difference	11.9%	—	33.8%	—
95% confidence limits	−2.9, 25.2	—	−5.6, 58.0	—

Note: Period 1 was 12 weeks in AC-052-351 and 16 weeks in AC-052-352.

* Proportion of patients improved compared with placebo.

ITT = intent-to-treat population, WHO = World Health Organization.

In Study AC-052-352, most patients (195 patients, 91.5%) entered the study in WHO class III, and 18 (8.5%) patients were classified as class IV. After 16 weeks of treatment, 36.1% of patients on bosentan had improved to class II, including three patients who entered as class IV (Table 12 and Figure 19). Three (2.1%) patients on bosentan were able to improve to class I from class III at entry. This is in contrast to the smaller proportion of patients on placebo who were able to reach class II from class III, and no placebo patients attained class I. The distribution of patients among the WHO functional classes at the end of Period 1 was similar between the two bosentan dose groups. The substantial improvement in 9 of the 14 patients on bosentan who entered in the worst category (class IV) confirms the beneficial effects observed in the walk test results of this subpopulation with severe disease.

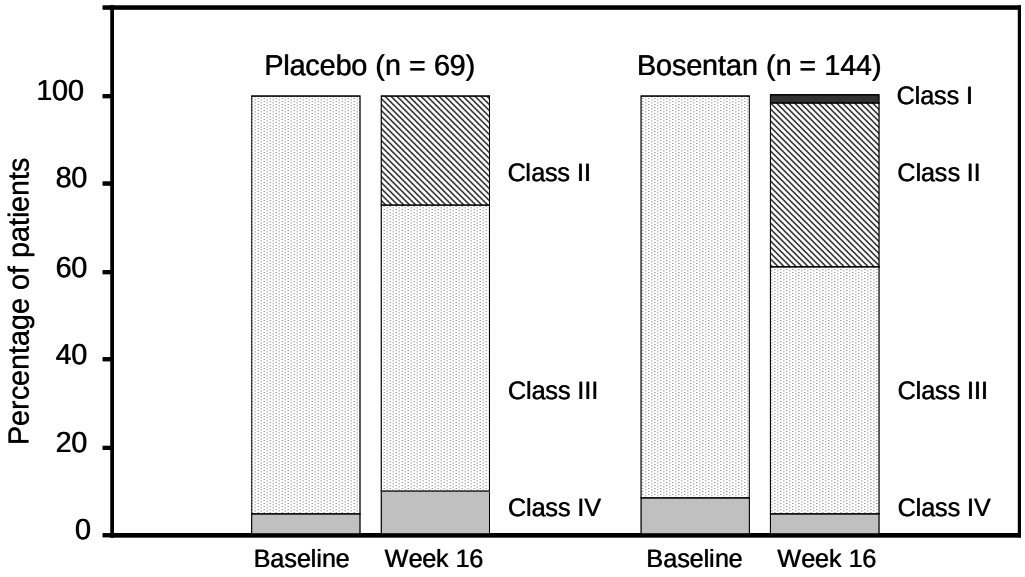
Table 12 WHO functional class: Change from baseline to Week 16 in Study AC-052-352, ITT population

(Table T11a / 04MAY01)

Treatment	N	n (%)	Baseline WHO class	Week 16 I No.	%	II No.	%	III No.	%	IV No.	%
Bosentan 125 mg	74	68 (91.9%)	III	2	2.7%	26	35.1%	38	51.4%	2	2.7%
		6 (8.1%)	IV	-		2	2.7%	2	2.7%	2	2.7%
Bosentan 250 mg	70	62 (88.6%)	III	1	1.4%	23	32.9%	37	52.9%	1	1.4%
		8 (11.4%)	IV	-		1	1.4%	4	5.7%	3	4.3%
All Bosentan	144	130 (90.3%)	III	3	2.1%	49	34.0%	75	52.1%	3	2.1%
		14 (9.7%)	IV	-		3	2.1%	6	4.2%	5	3.5%
Placebo	69	65 (94.2%)	III	-		19	27.5%	42	60.9%	4	5.8%
		4 (5.8%)	IV	-		-		2	2.9%	2	2.9%

ITT = intent-to-treat population, WHO = World Health Organization

Figure 19 Improvement in WHO functional class in Study AC-052-352, ITT population



6.5 Hemodynamic Effects

The effects of bosentan on pulmonary and cardiac function were evaluated in Study AC-052-351 using invasive hemodynamics assessed at baseline and after 12 weeks of oral treatment. The beneficial effects observed in central hemodynamic parameters support the observed clinical benefits of bosentan. After 12 weeks of controlled evaluation in Study AC-052-351, patients treated with bosentan showed consistent mean improvements in all parameters relative to baseline (Table 13 and Appendix 8), while those receiving placebo showed a clear worsening of hemodynamic status. Although no formal hypothesis testing was planned for these parameters, the importance of bosentan's effect is demonstrated by the significant reductions in both mean PAP and PVR, and the significant improvements in cardiac index, mean right atrial pressure (RAP) and PCWP compared with placebo when analyzed using the Student's t-test.

To test robustness of results, an exploratory, non-parametric analysis of hemodynamic parameters was performed using the Mann-Whitney U-test, and similar results were found (Table 13). Analyses using altered substitution rules (missing value replaced by last value carried forward rather than by the worst rank value when patients were prematurely withdrawn for reasons of death or worsening of pulmonary arterial hypertension without a premature withdrawal assessment) also showed statistically significant improvements in pulmonary and cardiac variables whether by Student's t-test ($p \leq 0.03$) or the Mann-Whitney U-test ($p < 0.02$).

These beneficial hemodynamic responses were not associated with excessive or rapid decreases in blood pressure, and no relevant effect on heart rate was observed in patients receiving bosentan (see Section 7.11).

Table 13 Change from baseline to Week 12 in central hemodynamic parameters in Study AC-052-351, ITT Population

Hemodynamic Parameter	Bosentan (n = 20)	Placebo (n = 10)	Treatment Difference	
			Mean (95% CL)	p-value[a]
Mean PAP (mmHg)				
Baseline Value	53.7 ± 3.0	55.7 ± 3.3		
Change from BL	–1.6 ± 1.2	5.1 ± 2.8	–6.7 (–11.9, -1.5)	0.0134 (0.0300)
% Change from BL	–2.5 ± 2.4	9.4 ± 5.0		
PVR (dyn·sec/cm^5)[b]				
Baseline Value	896 ± 97	942 ± 136		
Change from BL	–223 ± 56[c]	191 ± 74[c]	–415 (-608, -221)	0.0002 (0.0001)
% Change from BL	–19.9 ± 4.8	27.1 ± 10.7		
Cardiac index (l/min/m^2)				
Baseline	2.35 ± 0.16	2.48 ± 0.33		
Change from BL	0.50 ± 0.10[c]	–0.52 ± 0.15[c]	1.02 (0.65, 1.39)	<0.0001 (<0.0001)
% Change from BL	26.4 ± 5.7	–19.9 ± 5.9		
Mean RAP (mmHg)[b]				
Baseline Value	9.7 ± 1.3	9.9 ± 1.3		
Change from BL	–1.3 ± 0.9	4.9 ± 1.5[c]	–6.2 (-9.6, -2.7)	0.0010 (0.0010)
PCWP (mmHg)[b]				
Baseline Value	9.3 ± 0.6	8.3 ± 1.1		
Change from BL	0.1 ± 0.8	3.9 ± 1.8	–3.8 (-7.3, -0.3)	0.0353 (0.0270)

Values are mean ± standard error. One patient in each treatment group had no valid Week 12 assessment (out of allowable time window) and were not included in the analysis.

[a] Determined by Student's t-test (Mann-Whitney U-test).

[b] n =19 in bosentan group (Week 12 PCWP not available for one patient).

[c] A significant change from baseline (as determined by a 95% confidence interval that does not include zero change).

BL = baseline; CL = confidence limit; PAP = pulmonary arterial pressure; PCWP = pulmonary capillary wedge pressure; RAP = right atrial pressure; PVR = pulmonary vascular resistance.

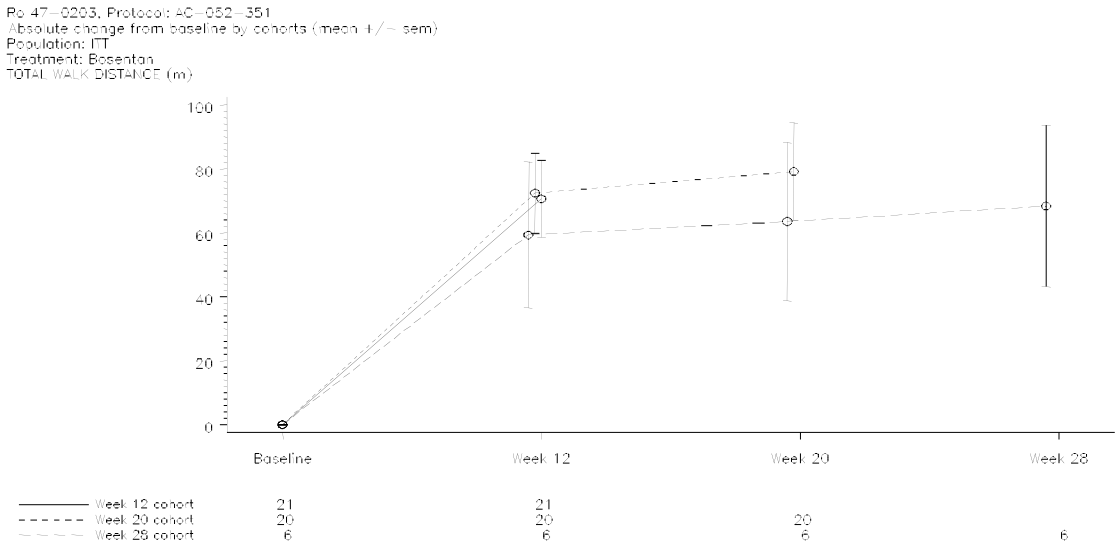
6.6 Long-term Effect

The demonstration of continued benefit with long-term treatment with bosentan is primarily based on the data obtained from patients who received treatment in Period 2 of Study AC-052-351 and from the 29 patients in the open-label extension study (AC-052-353). Twenty-eight of the patients in the extension study have been treated with bosentan for at least 1 year, and 7 have been treated for at least 1.5 years. During this time (up to the clinical cut off on 31 March 2001 or the 12-month visit, if later), only one patient (ex-placebo) has been withdrawn from the study (Day 105) and placed on epoprostenol treatment. Efficacy assessments in the extension trial included the 6-minute walk test at the Week 4 visit and the assessment of WHO functional class at each clinic visit.

6.6.1 Six-minute Walk Test

In patients treated for extended periods, the increase in walk distance was maintained during continued treatment, and no evidence for tolerance has been observed. In Study AC-052-351, patients were treated with bosentan for variable time periods up to 28 weeks. The cohort of patients who completed 28 weeks of treatment (n = 6) obtained an increase in walk distance similar to that obtained by the cohorts who were assessed up to Week 12 (n = 21) or up to Week 20 (n = 20), with no signs of a loss of effect (Figure 20). In Study AC-052-352, similar results were observed among the 48 patients (35 on bosentan and 13 on placebo) who entered Period 2. At the Week-28 assessment, mean ± SD changes from baseline was 43.0 ± 80.9 meters (95% CL of 15.3 and 70.8 meters) with bosentan and 5.8 ± 75.8 meters (95% CL of –40.1 and 51.6 meters) with placebo.

Figure 20 Walk test: Change from baseline to end of study in Study AC-052-351, ITT population



All 21 bosentan patients and 8 of 11 placebo patients in this study continued treatment in the extension study (AC-052-353). Three placebo patients in AC-052-351 were discontinued due to clinical worsening and were not eligible for the extension trial. At the Week 4 assessment in the extension study, ex-bosentan patients (n = 21) had a small mean ± standard error of the mean (SEM) increase of 3.0 ± 9.2 meters from the last value in the previous study, indicating continued benefit from treatment. For the six patients who had been treated with bosentan for 28 weeks in AC-052-351, this represented continued treatment for up to 32 weeks. Ex-placebo patients (n = 8) achieved an increase of 22.5 ± 14.3 meters from the last value in the previous study to the Week 4 assessment, which was consistent with the benefit obtained after 4 weeks of treatment with bosentan 62.5 mg b.i.d. in the placebo-controlled trials. At the Week 4 assessment, the

treatment in the previous placebo-controlled study was still blinded for 20 of the 21 ex-bosentan and for 6 of the 8 ex-placebo patients.

6.6.2 WHO Functional Class

Although all patients in the open-label extension study were considered WHO functional class III upon entry in the previous placebo-controlled study (AC-052-351), 44.8% of these patients were considered improved (i.e., 12 patients in class II and one in class I) after 6 months of treatment in the extension study. This proportion of improved patients remained stable at the 1-year assessment (11 patients in class II and one in class I). The one patient who was withdrawn from the study after about 3.5 months (Day 105) had deteriorated to class IV.

6.6.3 Survival

No patients in the open-label extension (AC-052-353) have died during the reported period. In order to put this in perspective, survival was evaluated in a cohort of matched patients (based on baseline hemodynamic measurements) derived from NIH registry data (prior to the availability of epoprostenol). Based on the model developed by Dr. Paul Levy (University of Illinois), it was expected that survival at 6 and 12 months would be 81% and 70%, respectively [14].

6.7 Conclusions

Compared with placebo, twice daily treatment with bosentan 125 mg or 250 mg was associated with:

- Statistically significant and clinically meaningful increases in walk distance (6-minute walk test), indicating improvement in exercise capacity. The improved walk distance during treatment with bosentan was maintained for at least 32 weeks, with no evidence for tolerance.
- Consistent improvements in walk distance in all subpopulations assessed, regardless of demographics, disease characteristics, and baseline parameters (e.g., baseline walk test and hemodynamics).
- Improvement of dyspnea during exercise which was achieved with a greater walk distance, suggesting symptom relief.
- A greater proportion of patients with improvement in WHO functional class, including in the worst cases (WHO class IV).
- A significantly lower risk of clinical worsening over the 28 weeks of treatment, which was already observed at the Week-16 assessment, suggesting that bosentan may modify the course of disease.
- Significant improvements in central hemodynamic parameters (pulmonary pressures, pulmonary vascular resistance, right atrial pressure, and cardiac index) with no associated increase in heart rate (assessed with bosentan 125 mg b.i.d. only).
- Sustained benefit in patients treated for at least 1 year.

The consistent improvements in all clinical and hemodynamic parameters induced by bosentan in patients with pulmonary arterial hypertension indicate that bosentan is an effective oral therapy that can bring to these patients improved exercise capacity and relief of symptoms and may modify the course of the disease.

7 SAFETY AND TOLERABILITY OF BOSENTAN

7.1 Overall Clinical Program for Bosentan

Safety data from the studies in pulmonary arterial hypertension (see Section 5.1) were integrated with the results from 7 therapeutic studies in other indications in order to provide a comprehensive assessment of the safety and tolerability of bosentan. These studies, listed in Table 14, were of ≥ 2 weeks duration and were conducted in patients with severe CHF (5 studies), systemic hypertension (one study) and SAH (one study). In all, the therapeutic safety database consists of 8 completed placebo-controlled trials and 3 open-label trials (2 of which are extensions of placebo-controlled trials and are currently ongoing). In addition, a placebo-controlled study in severe CHF (ENABLE, combined data from the identical studies AC-052-301 and AC-052-302) is currently being conducted and provides blinded safety data.

Table 14 Therapeutic studies in patients with other indications

Protocol (Report No.)	Study Objectives	Treatment Duration	Treatment*	No. of Patients in Safety Analysis
BC15064 † (B-165229)	Safety, hemodynamics and PK in patients with severe CHF (open-label and double-blind studies)	Part I (OL): 2 wks Part II (DB): 2 wks	Bosentan 500 mg b.i.d. oral Bosentan 1000 mg b.i.d. oral Placebo	7 24 12
NC15018 (B165231)	Safety and tolerability in patients with severe CHF	12 weeks	Bosentan 1000 mg b.i.d. Placebo	25 9
NC15462 REACH-1 (B-166849)	Efficacy, safety and PK in patients with severe CHF	26 weeks	Bosentan 125/250/500 mg b.i.d. Bosentan 250/500 mg b.i.d. Placebo	121 123 126
NC15464B (Interim Rept. B-00.030)	Long-term safety in patients with severe CHF (ongoing, open-label extension of NC15462)	Ongoing	Bosentan 125 mg b.i.d.	86
NC15020 (B-165230)	Efficacy, safety and dose range in patients with mild to moderate essential hypertension	4 weeks	Bosentan 100 mg q.d. Bosentan 500 mg q.d. Bosentan 1000 mg q.d. Bosentan 1000 mg b.i.d. Placebo	50 49 45 50 49
NN15031 (B-166850)	Safety, tolerability and PK/PD in SAH patients	2 weeks	Bosentan 500 mg t.i.d. Placebo (oral suspensions)	21 9
AC-052-301 AC-052-302 ENABLE (Interim Rept. B-00.029)	Efficacy and safety in patients with severe CHF (ongoing, still blinded)	≥ 9 months (mean of 18 mos)‡	Bosentan 62.5/125 mg b.i.d. Placebo	1613 (1:1 randomization)

* 500 mg = fixed dose; 125/250/500 mg = forced titration after 1 week of treatment; 62.5/125 mg = forced titration after 4 weeks of treatment.
† Parts I and II are counted as two independent trials, one open label and one double blind.
‡ The study will continue until the last patient has been treated for at least 9 months and until 600 deaths or hospitalizations for heart failure have occurred.
b.i.d. = twice daily; CHF = chronic heart failure; OL = open label; DB = double blind; PK/PD = pharmacokinetic/pharmacodynamic; q.d. = once daily; SAH = subarachnoid hemorrhage; t.i.d. = three times daily.

All available safety data from those studies completed as of the NDA clinical cut-off date (30 June 2000) are included in this report. Safety data from the since completed pivotal study in pulmonary arterial hypertension (AC-052-352) have been added. For the ongoing, open-label extension studies (AC-052-353 [see Section 5.3.2] and NC15464B) and the still blinded morbidity/mortality ENABLE study in patients with severe CHF, any additional data collected up to the most recent clinical cut-off dates have been included (Table 15).

Table 15 Dates for clinical cut off of safety assessments

Study	Safety Assessments	Cut-off Date
NC15464B	All safety parameters until the hand-over of the study from F. Hoffmann-La Roche to Actelion Pharmaceuticals (September 1999) and discontinuations, SAEs and deaths thereafter (up to the clinical cut-off)	31 December 2000
AC-052-353	All safety parameters	31 March 2001 or 12-month visit if later
ENABLE	Discontinuations, SAEs, deaths, clinical laboratory findings	31 December 2000

SAE = serious adverse event.

7.2 Study Designs and Methodology

In addition to the studies in pulmonary arterial hypertension (see Section 5.1), therapeutic studies with bosentan have been conducted in CHF, systemic hypertension, and SAH. Each of these studies is described below, and graphic displays of the design of these studies are provided in Appendix 9.

7.2.1 Studies in Chronic Heart Failure

Six trials (3 placebo-controlled, 2 open-label and one ongoing, blinded trial) have been or are being conducted in patients with severe CHF. The 3 completed placebo-controlled trials (BC15064 Part II, NC15018 and NC15462 [REACH-1]) were conducted in a total of 440 patients. The 2 open-label trials consist of one completed trial (BC15064 Part I) and one ongoing extension of the REACH-1 trial (NC15464B) conducted in a total of 93 patients. One placebo-controlled morbidity/mortality study (ENABLE, n = 1613) is currently being conducted.

Study BC15064 (Part I) preceded BC15064 (Part II) and enrolled seven patients with New York Heart Association (NYHA) class III or IV CHF who did not continue to Part II. Open-label bosentan 500 mg b.i.d. was administered for 14 days in combination with standard therapy for CHF, including digitalis, diuretics and ACE inhibitors.

Study BC15064 (Part II) was a double-blind, randomized (2:1), placebo-controlled, single-center, preliminary study in which 36 patients with NYHA class III or IV CHF received either bosentan 1000 mg b.i.d. (n = 24) or placebo (n = 12) for 14 days in combination with standard medication for CHF, including digitalis, diuretics and ACE inhibitors. Safety data were collected periodically throughout the trial and at the 1-week follow-up.

Study NC15018 was a double-blind, randomized (3:1), placebo-controlled, preliminary study in 34 patients with NYHA class III and IV CHF. Patients received oral bosentan 1000 mg b.i.d. (n = 25) or matching placebo (n = 9) for 12 weeks in addition to their standard therapy for CHF.

Study NC15462 (REACH-1) was a large, double-blind, randomized, placebo-controlled, multicenter trial in which 370 patients with severe CHF (NYHA class IIIB and IV) were randomized to placebo (n = 126) or one of two oral bosentan dosing regimens (slow titration 125/250/500 mg b.i.d., n = 121, or fast titration 250/500 mg b.i.d., n = 123). Planned treatment

duration was for 26 weeks. The main objective of this study was to evaluate the effects of bosentan on the clinical status of patients with severe CHF after 6 months of treatment. This study was stopped prematurely due to an increased incidence of elevated liver aminotransferases and because a strategic decision was made to continue the clinical program at a lower dose (125 mg b.i.d.).

Study NC15464B was an open-label, long-term extension study for patients with CHF who participated in Study NC15462 (REACH-1). Based on the observations in the REACH-1 trial, bosentan 125 mg b.i.d. was the target dose for the extension trial. The study recruited a total of 89 patients from NC14562 (86 patients actually started treatment, of whom 63 were formerly receiving bosentan and 23 placebo). Bosentan is being administered at a dose of 125 mg b.i.d., and long-term safety data are being collected.

ENABLE is a double-blind, randomized, placebo-controlled, morbidity/mortality study currently being conducted in patients with severe CHF (NYHA class IIIB/IV). A total of 1613 patients were enrolled and randomized to placebo or oral bosentan (1:1 randomization) given twice daily (target dose of 125 mg b.i.d.) for an estimated average treatment period of 18 months. Selected safety data, including deaths, SAEs, permanent discontinuations from study medication and selected laboratory parameters (i.e., liver function tests and hematological parameters) are presented for the study population where appropriate. However, because the data are still blinded, these safety data have not been combined with data from other studies.

7.2.2 Studies in Systemic Hypertension and Subarachnoid Hemorrhage

One placebo-controlled, dose-finding trial was conducted in patients with systemic hypertension, and one placebo-controlled trial was conducted in patients with SAH. The study in SAH was performed for the purpose of proof of concept.

Study NC15020 was a double-blind, parallel-group, placebo- and active-controlled (enalapril 20 mg/day) study of four dosages of bosentan (100, 500, 1000, 2000 mg/day) in patients with mild-to-moderate essential hypertension. A total of 293 patients were randomized approximately equally to each of the six treatment groups, and study drug was administered for 4 weeks. Safety assessments were made periodically during the study, and AEs, laboratory tests, and vital signs were also assessed at the follow-up visit, 1 week after cessation of treatment. The study was prematurely stopped by the sponsor to investigate reports of elevated liver enzymes after 293 of the planned 300 patients had been enrolled. For this integrated analysis, patients randomized to enalapril (n = 50) were not considered. Data from the remaining 243 patients were included in the integrated safety database.

Study NN15031 was a single-blind, placebo-controlled study of bosentan in severely ill patients with documented SAH. Patients received placebo or bosentan (oral suspension 500 mg every 8 hours) for 2 weeks in addition to standard care for SAH. Randomization was in a 3:1 ratio of bosentan (n = 21) to placebo (n = 9). In addition to periodic safety assessments during the treatment period, AEs and laboratory tests were collected for 2 weeks following cessation of trial treatment. The study was stopped after the 30 patients had been entered into the first phase of the

study because a strategic decision was made not to continue the development program in this indication. All 30 patients were included in the integrated safety database.

7.2.3 Analysis of Safety Data

For the integrated safety analysis, the safety population was defined as all patients who received at least one dose of study medication. "Study medication" included all investigational agents (including placebo) administered during the course of the study. In all studies, safety assessments included AEs, clinical laboratory tests, ECGs, vital signs, and premature discontinuation of study medication. In Study NC15464B, only discontinuations, SAEs and deaths were recorded after September 1999.

Data from the various studies were pooled in several ways in order to carefully assess the safety of bosentan over a broad spectrum of studies, patients, doses, and treatment durations. Descriptive summaries of the data, without statistical significance testing, were planned. The integrated analyses of safety data were performed on the following pools of studies:

– All studies of oral bosentan (11 studies)

– Randomized, double- or single-blind, placebo-controlled studies of oral bosentan (8 studies)

– Open-label, non-comparative studies of oral bosentan (3 studies)

– Total daily dose of oral bosentan:

  - Low dose: 100 mg/day
  - Doses evaluated in pulmonary arterial hypertension: 250–500 mg/day
  - High dose: 1000–1500 mg/day
  - Very high dose: 2000 mg/day

– Indication (pulmonary arterial hypertension, CHF, systemic hypertension, SAH)

– Planned treatment duration (≤ 12 weeks, > 12 weeks)

7.2.4 Presentation of Safety Data in this Report

The safety data for bosentan from all studies (integrated safety database of 30 June 2000 + Study AC-052-352 and up-dated data from ongoing studies) are evaluated in Sections 7.5 to 7.12. Although the safety data have been pooled and analyzed in a variety of ways, discussions in this document have focused on the safety data from the combined placebo-controlled studies in order to best illustrate the effect of bosentan treatment. Nearly all patients in the therapeutic trials participated in a placebo-controlled study, as only seven patients participated in a 14-day open-label trial (BC15064 Part I) that was not an extension of a placebo-controlled study. Data from specific studies or indications are presented when appropriate to demonstrate a specific finding. Within the pool of placebo-controlled studies, data from patients treated with the doses evaluated in pulmonary arterial hypertension (250 to 500 mg/day) are also discussed where appropriate in

order to illustrate bosentan's safety profile at the recommended dosage. Data from ENABLE are still blinded as to treatment and are presented only for selected safety parameters.

The safety of bosentan in pulmonary arterial hypertension was addressed by combining data from the two placebo-controlled studies, AC-052-351 and AC-052-352. Available long-term safety data in the extension trial (AC-052-353) are discussed separately because of the longer duration of treatment and lack of placebo control. Data from the exploratory study (BD14884) are also discussed separately because of the differences in study design, route of administration, dosage, and duration of treatment (see Appendix 1).

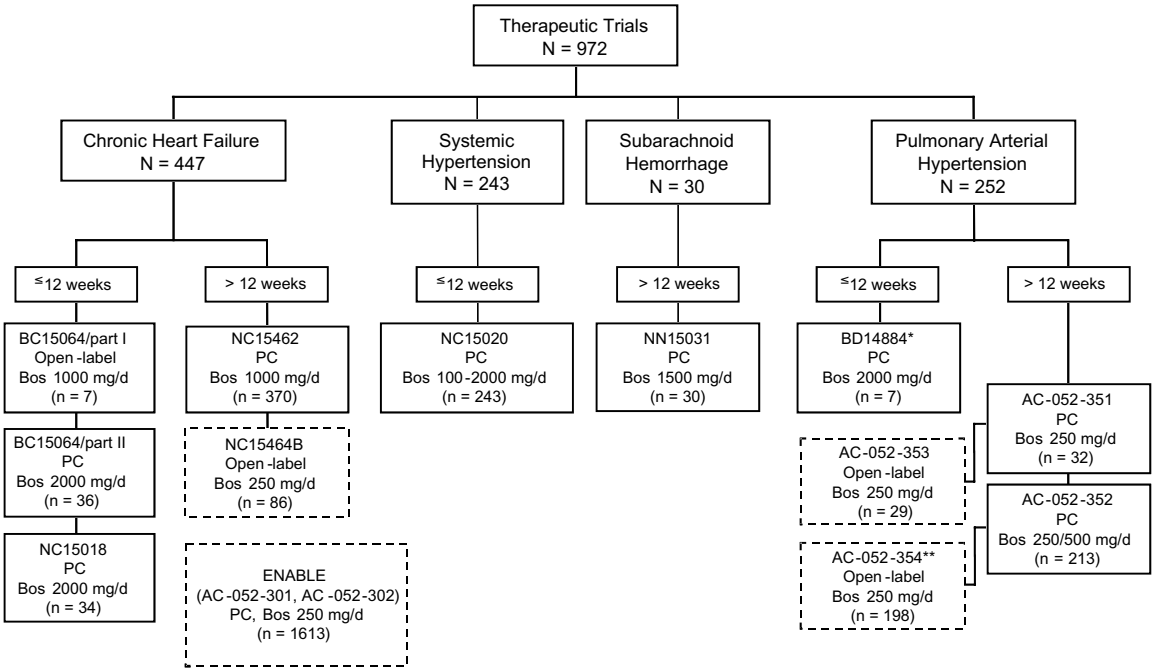
7.3 Overall Patient Population

7.3.1 Composition of Overall Database

The bosentan safety database includes 11 therapeutic studies of ≥ 2 weeks duration (Figure 21) with an admixture of patients (N = 972) representing four indications: pulmonary arterial hypertension (26%), CHF (46%), systemic hypertension (25%) and SAH (3%). Although only 26% of the patients in the database have pulmonary arterial hypertension, those with severe CHF (N = 447) have several pathophysiological characteristics that are similar, such as low cardiac output, high pulmonary pressures and right heart failure. The majority (85%) of these patients were from three studies, NC15462 in severe CHF (N = 370), AC-052-352 in pulmonary arterial hypertension (N = 213), and NC15020 in systemic hypertension (N = 243).

The bosentan dosages used cover a 20-fold range (100 mg/day to 2000 mg/day) and were administered for a variety of planned treatment durations (37% of patients n trial of ≤ 12 weeks and 63% in trials of > 12 weeks duration).

Figure 21 Therapeutic studies in the integrated safety database



* = Each of the 7 patients received bosentan 500 mg i.v. on Day 1.
** = Patients from the extension study AC-052-354 are not included in the safety analysis.
Bos = bosentan, PC = placebo-controlled.

The total numbers of patients in the various populations in the integrated safety database are given in Table 16. Excluding the 1613 patients in the still blinded ENABLE study, 715 patients have been treated with bosentan, and a total of 972 patients have participated in the clinical development program. All but 7 of the 972 patients participated in one of the 8 placebo-controlled studies, with 115 of these patients continuing treatment in one of the two open-label extension studies. The other seven patients participated in an open-label study (BC15064 Part I) that was not an extension of a placebo-controlled study.

Table 16 Summary of populations within the integrated database

	Placebo	Bosentan	All Patients
Patients in the 11 therapeutic trials	288	715*	972
Patients in the 8 placebo-controlled trials	288	677	965
Patients in the 3 open-label trials			
2 Extension trials (previous treatment)	31	84	115
1 Non-extension trial (BC15064 Part I)	—	7	7
Patients in the still blinded ENABLE study	1:1 randomization		1613

* Includes 677 bosentan-treated patients from placebo-controlled studies, 31 ex-placebo patients treated with bosentan in open-label extensions, and 7 bosentan-treated patients in the non-extension open-label trial.

7.3.2 Patients in Placebo-controlled Studies

7.3.2.1 Common Inclusion and Exclusion Criteria

Demographic selection criteria were similar in all studies. Patients' age at entry had to be ≥ 18 years in all studies except AC-052-352 (≥ 12 years). Male and female patients were recruited in all of the studies. Women were to be either surgically sterile, postmenopausal, or if of childbearing potential, must have been practicing an acceptable method of contraception.

The continued use of standard medications for the indication (pulmonary arterial hypertension, CHF and SAH) was allowed during the trials. The exceptions were antihypertensive treatment in the hypertension trial (NC15020) and epoprostenol therapy in patients with pulmonary arterial hypertension.

Patients with CHF had to have an ejection fraction < 35% (≤ 30% in Studies NC15018 and BC15064) and NYHA class III/IV (IIIB/IV in Studies NC15462, AC-052-301 and AC-052-302).

Common exclusion criteria included:

- Presence of clinically significant hepatic, pulmonary*, hematological, gastrointestinal, neurological†, renal, endocrine (except for well controlled diabetes mellitus and hypothyroidism), and autoimmune disease‡.
- Patients with known allergy or contraindication to any of the study drugs.
- Patients with a history or concomitant diagnosis of drug or alcohol abuse.
- Patients who were unable or unwilling to abide reliably with the requirements of the protocol.
- Patients who received an investigational compound within 1 month of study start.
- Patients previously exposed to bosentan.

*Except studies in patients with pulmonary arterial hypertension.
†Except for NN15031.
‡Except for BD14484, AC-052-351, AC-052-352, and AC-052-353.

Some of the more specific exclusion criteria are summarized in Appendix 10.

Several drugs or groups of drugs were not allowed to be administered during certain of the studies (Appendix 11). The decision to exclude specific drugs was based on the possibility of their interference with the ability to assess the efficacy or the safety of bosentan. For example, anti-hypertensive drugs in the hypertension trial and anti-arrhythmic and oral inotropic agents (except digoxin) known to be associated with safety concerns. In addition, several drugs were not allowed to be administered concomitantly with bosentan because of possible drug–drug interactions (e.g., cyclosporine A and glibenclamide [glyburide]).

Each study included additional specific inclusion and exclusion criteria related to the specific population studied, which can be found in the individual Final Study Reports.

7.3.2.2 Demographic and Baseline Characteristics

The overall population of patients in the placebo-controlled trials include all but seven patients in the database. Demographic and baseline clinical characteristics of these patients are summarized in Table 17. The diversity of the population can be seen in the distribution within each study descriptor (indication, planned treatment duration, and target dose).

Table 17 Demographic and baseline characteristics of patients in placebo-controlled studies, safety population

(Table T01_all_all_all / 17MAY01)
Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031

	Placebo N=288	Bosentan N=677
SEX		
n	288	677
Males	163 56.6%	413 61.0%
Females	125 43.4%	264 39.0%
AGE (years)		
n	288	677
Mean	57.3	57.3
SD	14.1	13.4
Stderr	0.8	0.5
Median	59.0	59.0
Q1 , Q3	49.0 , 67.0	48.0 , 67.0
Min , Max	12 , 89	13 , 90
WEIGHT (kg)		
n	279	650
Mean	77.1	77.8
SD	15.3	16.8
Stderr	0.9	0.7
Median	76.2	76.4
Q1 , Q3	67.0 , 86.7	66.4 , 87.0
Min , Max	33 , 127	33.7 , 161.9
LOCATION		
n	288	677
US	91 31.6%	188 27.8%
Non-US	197 68.4%	489 72.2%
RACE		
n	288	677
BLACK	10 3.5%	38 5.6%
CAUCASIAN	259 89.9%	599 88.5%
OTHER	19 6.6%	40 5.9%
INDICATION		
n	288	677
CHF	147 51.0%	293 43.3%
HTN	49 17.0%	194 28.7%
PAH	83 28.8%	169 25.0%
SAH	9 3.1%	21 3.1%
PLANNED DURATION		
n	288	677
12 weeks or less	82 28.5%	268 39.6%
More than 12 weeks	206 71.5%	409 60.4%
TREATMENT DOSE		
n	288	677
Placebo	288 100%	-
Bosentan 100 mg/d	-	50 7.4%
Bosentan 250-500 mg/d	-	214 31.6%
Bosentan 1000-1500 mg/d	-	310 45.8%
Bosentan 2000 mg/d	-	103 15.2%

CHF = chronic heart failure, HTN = hypertension, PAH = pulmonary arterial hypertension,
Q1 = first quartile, Q3 = third quartile, SD = standard deviation, SAH = subarachnoid hemorrhage,
Stderr = standard error.

7.3.2.3 Premature Discontinuations

In the placebo-controlled studies, similar proportions of bosentan- and placebo-treated patients were withdrawn from the studies (Table 18). A greater proportion of patients on bosentan than

on placebo were withdrawn because of an AE, and this difference was largely due to the greater proportion of patients withdrawn because of an abnormal liver function test.

Table 18 Premature discontinuations in placebo-controlled studies, safety population

	Placebo (N = 288)	Bosentan (N = 677)
Administrative / Other [n (%)]	5 (1.7)	15 (2.2)
Patient's decision	4 (1.4)	13 (1.9)
Worsening of PAH condition	11 (3.8)	15 (2.2)
Death	12 (4.2)	22 (3.3)
Due to an adverse event*	22 (7.6)	70 (10.3)
Abnormal LFT	2 (0.7)	28 (4.1)
All withdrawals	54 (18.8)	135 (19.9)

LFT = liver function test, PAH = pulmonary arterial hypertension.

* Some patients withdrawn for worsening of their condition were also withdrawn because of an adverse event, but are not included in this analysis.

7.4 Exposure

The exposure to bosentan in the integrated database is given in Table 19 and illustrated in Figure 22. Most patients received treatment in 4-week, 12-week or up to 28-week studies, and some patients in the open-label studies have received bosentan for up to about 3.5 years. Mean exposure (± SD) in open-label trials alone was 495 ± 381 days (range 4 to 1139 days) as of the clinical cut-off dates.

Table 19 Exposure to bosentan in all studies, safety population

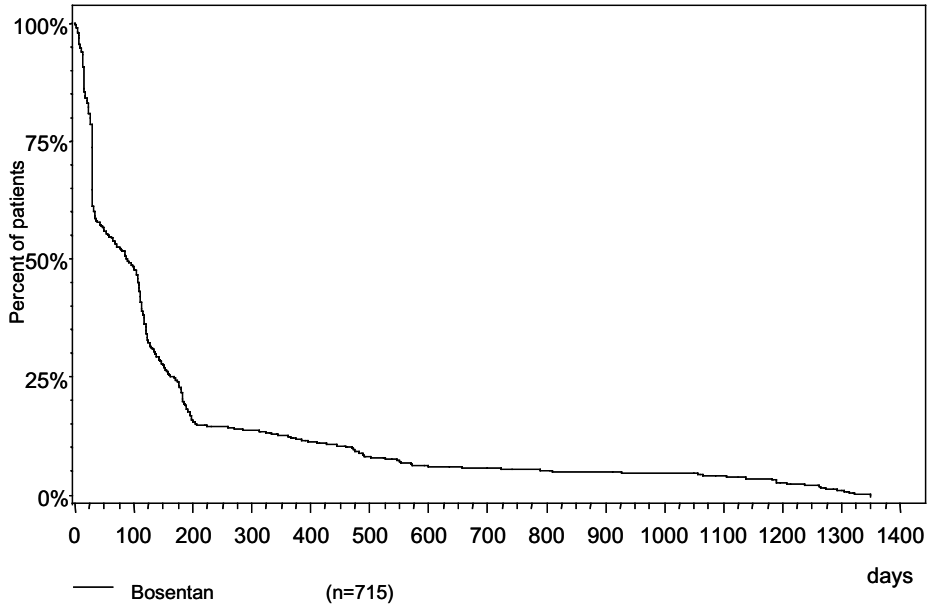
Table exp04 / 05JUL01)
Protocols: AC-52351 AC-52352 AC-52353 BC-15064(I) BC-15064(II)
BD-14884 NC-15018 NC-15020 NC-15462 NC-15464 NN-15031

	All patients N=715
Number of patients	
n	715
At least 4 weeks	526 73.6%
At least 3 months	352 49.2%
At least 6 months	141 19.7%
At least 1 year	88 12.3%
Exposure (days)	
n	715
Mean	168
SD	271
Stderr	10
Median	87
Min , Max	1 , 1349

SD = standard deviation, Stderr = standard error

Figure 22 Duration of exposure in all studies, safety population

Ro 47-0203, Protocol: AC-52351 AC-52352 AC-52353 BC-15064(I) BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NC-15464 NN-15031
FIGURE 1b Exposure to Bosentan
Population: Safety - All studies of oral Bosentan (including Open Label)



The overall exposure to study treatment in placebo-controlled studies is shown in Table 20, and treatment durations are illustrated in Figure 23. The mean exposure to bosentan was about 12 weeks.

Table 20 Exposure to study treatment in placebo-controlled studies, safety population

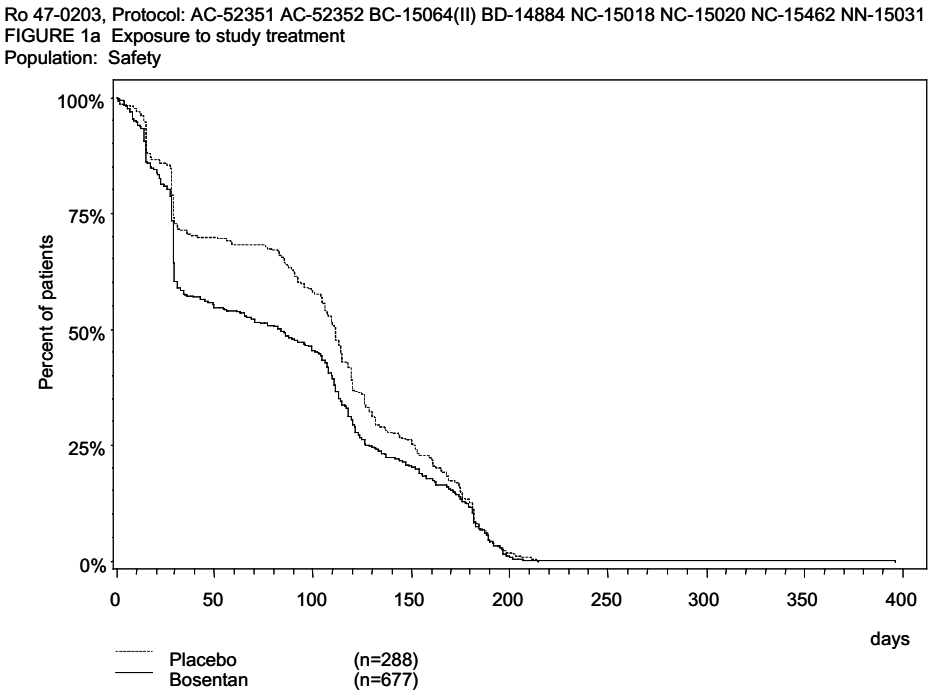
(Table exp01 / 08JUN01)
Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031

	Placebo N=288	Bosentan N=677
Exposure (days)		
n	288	677
Mean	101	85
SD	61	64
Stderr	4	2
Median	112	84
Min , Max	1 , 214	1 , 207*

* Excluding one patient with data entry error of 396 days.
SD = standard deviation, Stderr = standard error.

The exposure among bosentan- and placebo-treated patients in the placebo-controlled studies appears to differ, but this apparent difference is a result of the unequal numbers of patients in bosentan (n = 194) and placebo (n = 49) groups who completed scheduled treatment at the end of the 4-week study in hypertension (NC15020).

Figure 23 Duration of exposure in placebo-controlled studies, safety population



The exposure to bosentan in studies conducted in pulmonary arterial hypertension has been presented in Section 5.6.

At the clinical cut-off date, mean (± SD) exposure in the ongoing, still blinded ENABLE study in 1613 patients with severe CHF was 321 ± 119 days. At that time, 86% of patients had received at least 6 months of treatment and 39% had received at least 12 months.

7.5 Clinical Adverse Events

The pool of patients in placebo-controlled studies was evaluated in order to identify those events whose frequencies were higher with bosentan than with placebo (a difference of ≥ 2%) and were increased with the dose of bosentan. Specific AEs were identified as either one of the more frequent treatment-related AEs or of special interest and were analyzed in depth.

7.5.1 All Placebo-controlled Studies

A summary of the most frequent (≥ 3%) AEs in placebo-controlled studies (Table 21) reveals that only flushing (placebo-subtracted incidence of 4.9%), leg edema (3.3%), abnormal hepatic function (3.8%), headache (3.0%), and anemia (2.4%) were more frequent with bosentan than with placebo. In contrast, cardiac failure, nausea, and chest pain were more frequent on placebo. A complete summary of all AEs in the 8 placebo-controlled studies can be found in Appendix 12.

Table 21 Most frequent adverse events (≥ 3%) in placebo-controlled studies

(Table T31f_3 / 17MAY01)
Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031

Body system / Adverse event	Placebo N=288 No.	Placebo %	Bosentan N=677 No.	Bosentan %
ALL BODY SYSTEMS				
Total pts with at least one AE	220	76.4%	529	78.1%
Total number of AEs	840		1591	
CARDIAC FAILURE NOS	64	22.2%	120	17.7%
HEADACHE NOS	37	12.8%	107	15.8%
DIZZINESS (EXC VERTIGO)	39	13.5%	80	11.8%
HYPOTENSION NOS	22	7.6%	46	6.8%
FLUSHING	5	1.7%	45	6.6%
HEPATIC FUNCTION ABNORMAL NOS	6	2.1%	40	5.9%
UPPER RESPIRATORY TRACT INFECTION NOS	18	6.3%	32	4.7%
OEDEMA LOWER LIMB	4	1.4%	32	4.7%
NAUSEA	30	10.4%	31	4.6%
DIARRHOEA NOS	18	6.3%	30	4.4%
CHEST PAIN NEC	20	6.9%	27	4.0%
DYSPNOEA NOS	14	4.9%	26	3.8%
COUGH	13	4.5%	26	3.8%
NASOPHARYNGITIS	10	3.5%	23	3.4%
ANAEMIA NOS	3	1.0%	23	3.4%

Note: Only treatment-emergent AEs (including unrelated) are included.
Only AEs with an incidence >3% before rounding are included.
AE = adverse event, NEC = not elsewhere classified, NOS = not otherwise specified.

In order to assess potential pro-ischemic effects, the preferred terms of angina pectoris, chest pain (not musculo-skeletal), and chest pain cardiac were combined. In this analysis, the incidence was

higher on placebo than on bosentan (7.6% and 5.9%, respectively), indicating that treatment with bosentan is not associated with pro-ischemic effects.

Leg edema was further assessed by combining the preferred terms of edema lower limb, edema, and edema peripheral. Overall, the incidence of these events was greater in patients on bosentan than in those on placebo (7.4% and 2.8%, respectively). The incidence of these events tended to be dose related, and the difference was apparent in the doses evaluated in pulmonary arterial hypertension (12.6% and 5.4%, respectively).

Because of the increased incidence of abnormal hepatic function, the incidences of abdominal pain, nausea and vomiting were more carefully scrutinized. The incidences of these AEs were consistently higher among placebo-treated patients than bosentan-treated patients, whether evaluation was in all placebo-controlled studies, in patients receiving the doses evaluated in pulmonary arterial hypertension, or in patients with pulmonary arterial hypertension.

7.5.2 Effect of Dose

The incidence of AEs in placebo-controlled studies has been further assessed by bosentan dose in order to reveal any possible dose relationships. Each dose group was compared with its matching placebo controls (i.e., patients on placebo in the same studies). When AEs were categorized by dose, it appeared that all five AEs that were more frequent among the bosentan-treated patients in the pool of placebo-controlled studies were also dose related. Furthermore, in the dose-finding study in patients with systemic hypertension (NC15020, Table 22), the individual AEs that occurred more frequently on bosentan than on placebo and appeared to be dose related were headache, flushing and leg edema, though the incidence of headache was greater on bosentan than on placebo only at doses ≥ 1000 mg/day. Because abnormal liver function and anemia are based on laboratory tests, the actual occurrences of these events are likely to have been under-reported as AEs, and these effects of bosentan treatment are described in detail in Sections 8 and 9, respectively.

Table 22 NC15020: Most frequent (in ≥ 3 patients) adverse events in the dose-finding study in systemic hypertension, safety population

(Table T31e_htn_3 / 17MAY01)

Body system / Adverse event	Placebo	Bosentan 100 mg/d	Bosentan 250-500 mg/d	Bosentan 1000-1500 mg/d	Bosentan 2000 mg/d	Bosentan (all)
	N=49	N=50	N=49	N=45	N=50	N=194
	No. %	No. %	No. %	No. %	No. %	No. %
ALL BODY SYSTEMS						
Total pts with at least one AE	28 57.1%	21 42.0%	24 49.0%	24 53.3%	28 56.0%	97 50.0%
Total number of AEs	52	43	48	34	65	190
HEADACHE NOS	10 20.4%	7 14.0%	7 14.3%	10 22.2%	13 26.0%	37 19.1%
FLUSHING	-	3 6.0%	6 12.2%	4 8.9%	9 18.0%	22 11.3%
OEDEMA LOWER LIMB	-	1 2.0%	1 2.0%	2 4.4%	8 16.0%	12 6.2%
INFLUENZA	6 12.2%	3 6.0%	-	2 4.4%	5 10.0%	10 5.2%
COUGH	1 2.0%	2 4.0%	3 6.1%	-	2 4.0%	7 3.6%
DIZZINESS (EXC VERTIGO)	2 4.1%	4 8.0%	1 2.0%	-	1 2.0%	6 3.1%
OEDEMA NOS	-	1 2.0%	3 6.1%	-	2 4.0%	6 3.1%
RHINITIS NOS	1 2.0%	-	3 6.1%	2 4.4%	-	5 2.6%
FACE OEDEMA	-	1 2.0%	1 2.0%	-	3 6.0%	5 2.6%

Note: Only treatment-emergent AEs (including unrelated) are included.
AE = adverse event, NOS = not otherwise specified.

In patients receiving the doses evaluated in pulmonary arterial hypertension (250–500 mg/day), the most frequent AEs with a greater incidence in the bosentan- than the placebo-treatment group were flushing (placebo-subtracted incidence of 6.7%), abnormal hepatic function (4.9%), nasopharyngitis (3.7%), leg edema (3.4%), edema (3.1%), dyspepsia (2.9%), and hypotension (2.8%, see Appendix 13). In contrast, AEs with a greater incidence on placebo than on bosentan were dizziness, nausea, aggravated pulmonary hypertension, dyspnea, influenza, and abdominal pain.

7.5.3 Studies in Pulmonary Arterial Hypertension

Patients who were enrolled in one of the two pivotal trials (AC-052-351 and AC-052-352) comprise 77% of the 214 patients in the safety database who received the doses evaluated in pulmonary arterial hypertension (250–500 mg/day) in placebo-controlled trials. It is therefore not surprising that the AE profile in patients with pulmonary arterial hypertension is similar to that observed among patients who received these doses.

A summary of the most frequent (≥ 3%) AEs in placebo-controlled studies in pulmonary arterial hypertension (Table 23) reveals that abnormal hepatic function (placebo-subtracted incidence of 6.0%), dyspepsia (4.2%), flushing (4.1%), pruritus (3.6%), palpitations (3.5%), nasopharyngitis (3.4%), leg edema (2.9%), hypotension (2.9%), and fatigue (2.3%) were more frequent with bosentan than with placebo. Although apparently different between treatment groups, the incidences of dyspepsia, pruritus, palpitations, and fatigue were less than 5% among bosentan-treated patients. In contrast, dizziness, nausea, aggravated pulmonary hypertension, cough, dyspnea, influenza, abdominal pain, and limb pain were more frequent on placebo.

Table 23 Most frequent (≥ 3%) adverse events in placebo-controlled studies in pulmonary arterial hypertension, safety population

(Table T31_12y / 21MAY01)
Protocols: AC-52351 AC-52352

Body system / Adverse event	Bosentan 250 mg/d N=95 No.	%	Bosentan 500 mg/d N=70 No.	%	Bosentan (all) N=165 No.	%	Placebo N=80 No.	%
ALL BODY SYSTEMS								
Total pts with at least one AE	90	94.7%	66	94.3%	156	94.5%	75	93.8%
Total number of AEs	317		257		574		316	
HEADACHE NOS	20	21.1%	16	22.9%	36	21.8%	16	20.0%
UPPER RESPIRATORY TRACT INFECTION NOS	11	11.6%	9	12.9%	20	12.1%	9	11.3%
NASOPHARYNGITIS	10	10.5%	8	11.4%	18	10.9%	6	7.5%
DIZZINESS (EXC VERTIGO)	10	10.5%	7	10.0%	17	10.3%	13	16.3%
FLUSHING	9	9.5%	6	8.6%	15	9.1%	4	5.0%
NAUSEA	9	9.5%	5	7.1%	14	8.5%	11	13.8%
HEPATIC FUNCTION ABNORMAL NOS	4	4.2%	10	14.3%	14	8.5%	2	2.5%
SYNCOPE	6	6.3%	7	10.0%	13	7.9%	7	8.8%
OEDEMA LOWER LIMB	8	8.4%	5	7.1%	13	7.9%	4	5.0%
BRONCHITIS NOS	7	7.4%	5	7.1%	12	7.3%	7	8.8%
DIARRHOEA NOS	5	5.3%	7	10.0%	12	7.3%	6	7.5%
HYPOTENSION NOS	6	6.3%	5	7.1%	11	6.7%	3	3.8%
PULMONARY HYPERTENSION NOS AGGRAVATED	6	6.3%	4	5.7%	10	6.1%	16	20.0%
ARTHRALGIA	7	7.4%	3	4.3%	10	6.1%	5	6.3%
COUGH	5	5.3%	4	5.7%	9	5.5%	8	10.0%
DYSPNOEA NOS	4	4.2%	5	7.1%	9	5.5%	8	10.0%
CHEST PAIN NEC	6	6.3%	3	4.3%	9	5.5%	5	6.3%
SINUSITIS NOS	7	7.4%	2	2.9%	9	5.5%	4	5.0%
EPISTAXIS	5	5.3%	3	4.3%	8	4.8%	5	6.3%
RESPIRATORY TRACT INFECTION NOS	4	4.2%	4	5.7%	8	4.8%	4	5.0%
PALPITATIONS	5	5.3%	3	4.3%	8	4.8%	1	1.3%
INFLUENZA	2	2.1%	5	7.1%	7	4.2%	5	6.3%
OEDEMA NOS	4	4.2%	3	4.3%	7	4.2%	2	2.5%
DYSPEPSIA	4	4.2%	3	4.3%	7	4.2%	-	
ABDOMINAL PAIN NOS	3	3.2%	3	4.3%	6	3.6%	5	6.3%
BACK PAIN	4	4.2%	2	2.9%	6	3.6%	4	5.0%
URINARY TRACT INFECTION NOS	3	3.2%	3	4.3%	6	3.6%	4	5.0%
FATIGUE	2	2.1%	4	5.7%	6	3.6%	1	1.3%
PRURITUS NOS	4	4.2%	2	2.9%	6	3.6%	-	
PAIN IN LIMB	2	2.1%	3	4.3%	5	3.0%	6	7.5%
MUSCLE CRAMPS	4	4.2%	1	1.4%	5	3.0%	3	3.8%
DRY MOUTH	3	3.2%	2	2.9%	5	3.0%	1	1.3%
PNEUMONIA NOS	2	2.1%	3	4.3%	5	3.0%	1	1.3%

Note: Only AEs (including unrelated) with onset from start of treatment to 1 calendar day after end of treatment are included.
AE = adverse event, NEC = not elsewhere classified, NOS = not otherwise specified.

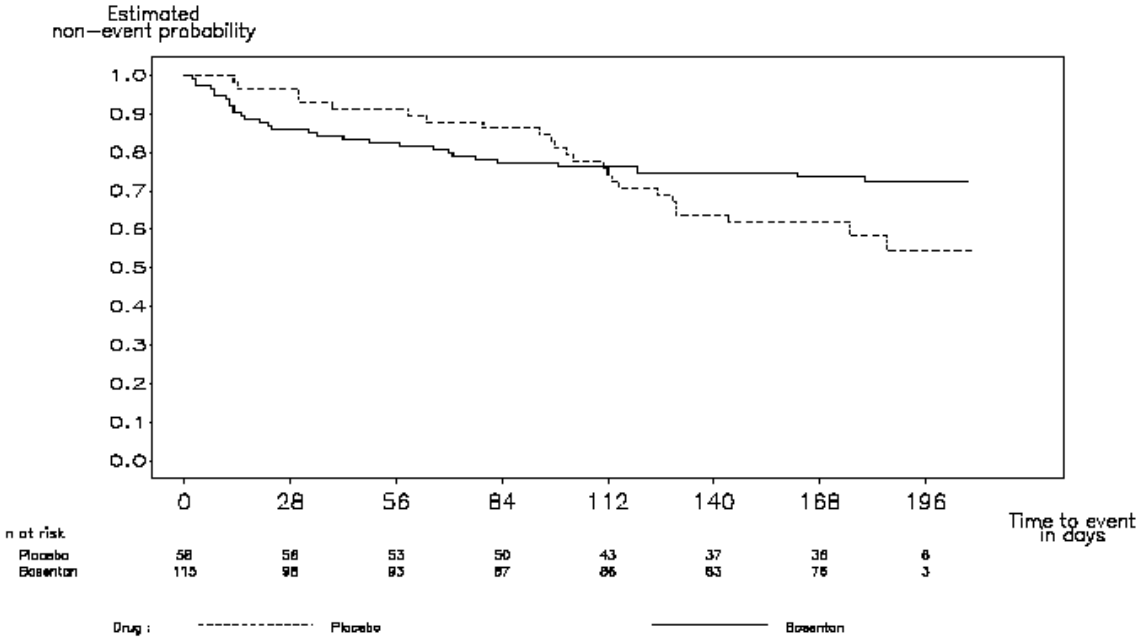
Open-label extension study (AC-052-353). Up to the clinical cut-off date, patients in this study have had at least 1 year of bosentan treatment, and nearly all patients (93.1%) have reported at least one AE. The AEs reported for at least 5 of the 29 patients were aggravated pulmonary arterial hypertension (7), dyspnea, sinusitis, upper respiratory tract infection (6 each), chest pain and fatigue (5 each). These AEs were not unexpected in this patient population over the course of a year, and only one patient was discontinued due to aggravated pulmonary hypertension.

7.5.4 Additional Observations

Cardiac failure. Among CHF patients randomized 6 months before the REACH-1 trial (NC15462) was stopped, the frequency of death or worsening CHF was found to be higher among

bosentan- than placebo-treated patients during the first month of treatment (Figure 24). Beyond the first 30 days, the frequency of these events increased in the placebo group, and at the time the study was stopped, the overall incidence was significantly ($p \leq 0.008$) lower among bosentan-treated patients. Most of the events in this analysis were hospitalizations for worsening heart failure, and there was no difference in the rate of death between the two groups.

Figure 24 NC15462: Kaplan-Meier estimates of time to death or worsening CHF, subpopulation randomized 6 months before the trial was stopped



The overall incidence of cardiac failure in all placebo-controlled studies was also greater in placebo-treated than in bosentan-treated patients (22.2% vs 17.7%, respectively). However, these numbers largely reflect the results of the REACH-1 study (NC15462) as 88% of all reports of cardiac failure were in this study. In REACH-1, the increased risk of hospitalization for heart failure during the first month of bosentan treatment appeared to be related to the starting dose of the drug (125 and 250 mg b.i.d.) and the speed of up-titration. The incidence was greater among patients who started at the 250 mg b.i.d. dose and were up-titrated to 500 mg b.i.d. after 1 week than in those who started at 125 mg b.i.d. and were up-titrated to 250 mg and 500 mg b.i.d. at weekly intervals.

As a result of these observations, the starting dose in the recent trials in pulmonary arterial hypertension and CHF was 62.5 mg b.i.d., and up-titration to 125 or 250 mg b.i.d. occurred after 4 weeks. No reports of cardiac failure during the first month of bosentan treatment occurred in the two pivotal studies in pulmonary arterial hypertension, supporting the conclusions from the REACH-1 trial (NC15462).

Rebound. Withdrawal effects could be assessed in three studies in which AEs were collected for 1 week following study drug discontinuation. No evidence of a withdrawal effect was observed in the 194 bosentan-treated patients in the dose-finding study in systemic hypertension (NC15020) or in the 49 CHF patients receiving very high doses of bosentan (2000 mg/day, BC15064 Part I and NC15018). Relevant events in the hypertension study were one case of coronary infarction in the low-dose group (100 mg/day) and one case of headache in the high-dose group (1000 mg/day). In the CHF studies, only two relevant events (coronary infarction and cardiac failure) were reported after treatment cessation, one in each study, even though patients in these trials were receiving very high doses of bosentan (2000 mg/day).

At present, there is insufficient experience in patients with pulmonary arterial hypertension to adequately assess the potential for rebound. Switching patients from the target dose (125 or 250 mg b.i.d.) to a lower dose (62.5 mg b.i.d.) in the transition to the open-label extension trials, resulted in reports of a worsening clinical condition for some patients. It is not clear if this was due to rebound or just loss of efficacy because of the dose reduction. However, as stopping other treatments in patients with pulmonary arterial hypertension (e.g., calcium channel blockers and epoprostenol) has been associated with a rebound effect, it is also possible that this could occur with cessation of bosentan treatment. Therefore, in Study AC-052-352 it was planned that treatment be stopped gradually, if possible (i.e., dose reduction to half the target dose for 3 to 7 days before full discontinuation).

7.6 Deaths

Of the 715 patients who have received bosentan in the completed placebo-controlled studies and the ongoing long-term, open-label extensions, there have been a total of 58 (8.1%) deaths, 10 of which occurred more than 28 days after treatment cessation. Most of these deaths (53 of 58 cases) occurred in patients with severe heart failure (NYHA class IIIB/IV) enrolled in the REACH-1 trial (NC15462) and its extension (NC15464B). In this ongoing, extension trial (which started in November 1998), a total of 27 (31.4%) patients have died as of the clinical cut-off date, primarily from cardiovascular events that are expected in this patient population. In the entire development program for bosentan, one additional death (due to bronchopneumonia) occurred in a SAH pharmacology study (BD14473E) 11 days after an i.v. infusion of 500 mg of bosentan.

7.6.1 All Placebo-controlled Studies

In the placebo-controlled trials, the proportions of patients who died were similar between treatment groups (4.6% of bosentan-treated and 5.2% of placebo-treated patients, Table 24). Eight of these deaths (5 in bosentan and 3 in placebo groups) occurred more than 28 days after treatment cessation, and if excluded, the overall incidences of death would be 3.8% and 4.2%, respectively. As previously indicated, most of these deaths occurred in the REACH-1 trial (NC15462) in CHF (in 26/244 patients on bosentan and 11/126 on placebo). In this study, all deaths among bosentan-treated patients were due to cardiovascular events (sudden death, cardiac failure, cardiac arrest, myocardial infarction, each in 3 or 4 patients), and none would be considered unexpected in this patient population. Sudden death (4 patients) was the most frequent reason among placebo-treated patients. Sixteen of the deaths (11 in bosentan and 5 in

placebo groups) occurred more than 1 week after discontinuation of study medication. Kaplan-Meier estimates of survival demonstrate the similarity between treatment groups (Figure 25).

A single death (hydrocephalus acquired, infarct) occurred in the SAH study (NN15031), and the remaining 6 deaths (4/144 patients on bosentan and 2/69 on placebo) occurred in Study AC-052-352 in pulmonary arterial hypertension (see Section 7.6.2).

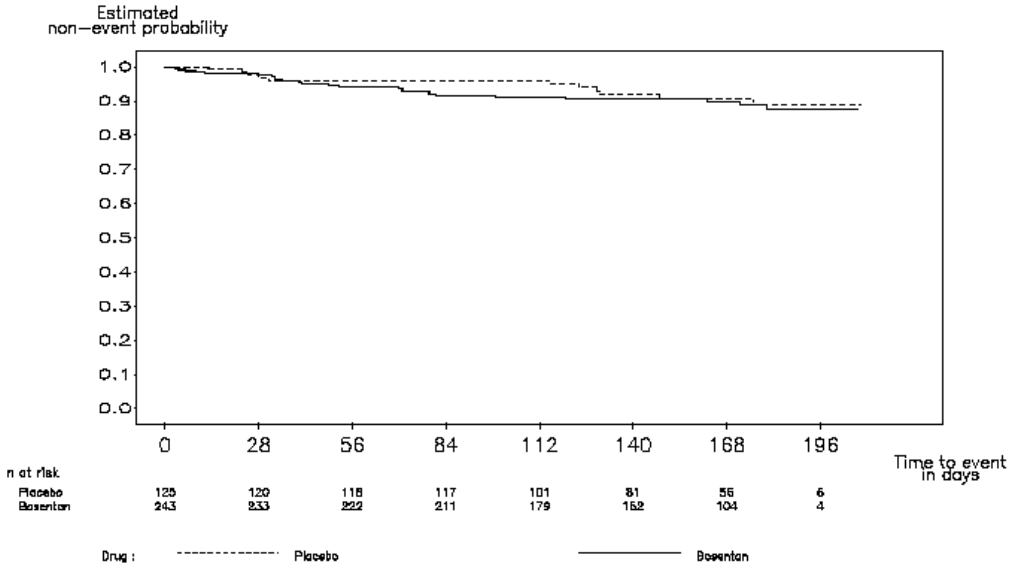
Table 24 Deaths in placebo-controlled studies, safety population

(Table T90f / 17MAY01)
Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031

Cause of death	Placebo N=288 No.	%	Bosentan N=677 No.	%
Total pts with at least one cause*	15	5.2%	31	4.6%
SUDDEN DEATH UNEXPLAINED	5	1.7%	3	0.4%
CARDIAC FAILURE NOS	1	0.3%	6	0.9%
CARDIAC ARREST	-		3	0.4%
CARDIOGENIC SHOCK	1	0.3%	2	0.3%
CEREBROVASCULAR ACCIDENT NOS	1	0.3%	2	0.3%
MYOCARDIAL INFARCTION	-		3	0.4%
PULMONARY HYPERTENSION NOS AGGRAVATED	2	0.7%	-	
DEATH NOS	1	0.3%	1	0.1%
PULMONARY OEDEMA NOS	-		2	0.3%
RENAL FAILURE NOS	-		2	0.3%
SEPSIS NOS	1	0.3%	1	0.1%
ARRHYTHMIA NOS	1	0.3%	-	
CARDIO-RESPIRATORY ARREST	-		1	0.1%
CORONARY ARTERY DISEASE NOS	-		1	0.1%
HEPATORENAL SYNDROME	1	0.3%	-	
HYDROCEPHALUS ACQUIRED	-		1	0.1%
MALIGNANT HEPATIC NEOPLASM	1	0.3%	-	
MULTI-ORGAN FAILURE	-		1	0.1%
PNEUMONIA NOS	-		1	0.1%
PULMONARY HAEMORRHAGE	-		1	0.1%
PULMONARY THROMBOSIS	1	0.3%	-	
VENTRICULAR FIBRILLATION	-		1	0.1%

* patients could have more than one reason for death
NOS = not otherwise specified.

Figure 25 NC15462: Kaplan-Meier estimates of survival, safety population



7.6.2 Studies in Pulmonary Arterial Hypertension

As in the pool of all placebo-controlled studies, the proportions of patients in studies on pulmonary arterial hypertension who died were similar between treatments (2.4% on bosentan and 2.5% on placebo, Table 25). Two patients on placebo died of aggravated pulmonary arterial hypertension during the study. Deaths in the bosentan group were from cardiac failure (two patients), pulmonary hemorrhage, and pneumonia/sepsis. An additional death due to aggravated pulmonary arterial hypertension occurred 32 days after stopping treatment with bosentan 250 mg b.i.d. There were no deaths in Study AC-052-351, and none of the 29 patients in the open-label extension study (AC-052-353) have died (mean ± SD total exposure 460 ± 95 days; range 105 to 594 days).

Table 25 Deaths in placebo-controlled studies in pulmonary arterial hypertension, safety population

(Table T90f_12y / 21MAY01)
Protocols: AC-52351 AC-52352

Cause of death	Bosentan 250 mg/d N=95 No.	%	Bosentan 500 mg/d N=70 No.	%	Bosentan (all) N=165 No.	%	Placebo N=80 No.	%
Total pts with at least one cause	1	1.1%	3	4.3%	4	2.4%	2	2.5%
CARDIAC FAILURE NOS	1	1.1%	1	1.4%	2	1.2%	-	
PULMONARY HYPERTENSION NOS AGGRAVATED	-		-		-		2	2.5%
PNEUMONIA NOS	-		1	1.4%	1	0.6%	-	
PULMONARY HAEMORRHAGE	-		1	1.4%	1	0.6%	-	
SEPSIS NOS	-		1	1.4%	1	0.6%	-	

Note: All the deaths either during the study or within 28 days after stopping study treatment are included.
NOS = not otherwise specified.

7.6.3 ENABLE

In the ongoing, blinded ENABLE study in CHF patients, a total of 187 (11.6%) patients have died as of the clinical cut-off date (N = 1613, with a mean ± SD exposure of 321 ± 119 days), including one patient (arrhythmia) known to have been on placebo. Most of the events in these trials have been the result of cardiovascular events, with cardiac failure (4.2% of patients) and sudden death (2.0% of patients) being the most frequent. None of the deaths was unexpected in this patient population.

7.7 Serious Adverse Events

Serious adverse events occurring in the therapeutic trials either during the study or within 28 days following the end of study treatment are summarized. In several studies, selected AEs normally considered serious were censored because they were expected in the specific patient population (e.g., worsening heart failure in patients with CHF). These events were reported as AEs and should not have been reported as SAEs but sometimes were, despite protocol instructions. As a result, the occurrence of these events is under-reported in summaries of SAEs.

7.7.1 Placebo-controlled and Open-label Studies

In the controlled studies, a greater proportion of patients on placebo than on bosentan experienced an SAE. Most of these events were not unexpected in the studied patient populations and were related to the underlying diseases. The majority of patients experiencing an SAE came from the studies in severe CHF.

Table 26 Serious AEs (including unrelated) occurring in ≥ 0.5% of patients in placebo-controlled studies, safety population

(Table T21f / 17MAY01)
Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031

Body system / Adverse event	Placebo N=288 No.	%	Bosentan N=677 No.	%
ALL BODY SYSTEMS				
Total pts with at least one AE	51	17.7%	92	13.6%
Total number of AEs	95		135	
CARDIAC FAILURE NOS	4	1.4%	6	0.9%
PNEUMONIA NOS	1	0.3%	6	0.9%
ANAEMIA NOS	2	0.7%	5	0.7%
ATRIAL FIBRILLATION	2	0.7%	5	0.7%
DYSPNOEA NOS	2	0.7%	5	0.7%
CHEST PAIN NEC	4	1.4%	4	0.6%
RENAL FAILURE NOS	3	1.0%	4	0.6%
ABDOMINAL PAIN NOS	1	0.3%	4	0.6%
MYOCARDIAL INFARCTION	1	0.3%	4	0.6%

Note: Only treatment-emergent serious AEs (including unrelated) are included.
AE = adverse event, NEC = not elsewhere classified, NOS = not otherwise specified.

No new pattern of events was observed in the ongoing, open-label studies, although a greater proportion of patients (45/122, 36.9%) were reported to have had a serious event than in placebo-controlled studies, which is most likely due to the longer duration of follow-up. The only SAEs experienced by more than two patients were pneumonia, cardiac failure, anemia, atrial fibrillation, cerebrovascular accident, and chest pain, all of which were expected in this population dominated by patients with severe CHF.

7.7.2 Studies in Pulmonary Arterial Hypertension

As in the larger database, a greater proportion of patients on placebo than on bosentan experienced an SAE in the pivotal studies in pulmonary arterial hypertension (21.3% and 17.6%, respectively). The only individual SAEs reported for more than two patients in any treatment group were anemia in 4 (2.4%) patients on bosentan and aggravated PHT in 3 (3.8%) patients on placebo (Table 27). In the extension study (AC-052-353), 5 SAEs have been reported in 4 patients (atrial fibrillation/gastroenteritis, hypercalcemia/hyperparathyroidism, chest pain, and palpitations), and all patients have been treated for at least 1 year.

Table 27 Serious AEs (including unrelated) occurring in ≥ 2 patients in placebo-controlled studies in pulmonary arterial hypertension (AC-052-351 and AC-052-352), safety population

(Table T21_12y / 21MAY01)
Protocols: AC-52351 AC-52352

Body system / Adverse event	Bosentan 250 mg/d N=95 No.	%	Bosentan 500 mg/d N=70 No.	%	Bosentan (all) N=165 No.	%	Placebo N=80 No.	%
ALL BODY SYSTEMS								
Total pts with at least one AE	14	14.7%	15	21.4%	29	17.6%	17	21.3%
Total number of AEs	17		24		41		33	
ANAEMIA NOS	3	3.2%	1	1.4%	4	2.4%	-	
CARDIAC FAILURE NOS	1	1.1%	1	1.4%	2	1.2%	2	2.5%
ABDOMINAL PAIN NOS	2	2.1%	-		2	1.2%	1	1.3%
PNEUMONIA NOS	-		2	2.9%	2	1.2%	1	1.3%
INTESTINAL OBSTRUCTION NOS	2	2.1%	-		2	1.2%	-	
BRONCHITIS NOS	-		1	1.4%	1	0.6%	2	2.5%
VOMITING NOS	1	1.1%	-		1	0.6%	2	2.5%
PULMONARY HYPERTENSION NOS AGGRAVATED	-		-		-		3	3.8%
NAUSEA	-		-		-		2	2.5%

Note: Only AEs (including unrelated) with onset from start of treatment to 28 calendar days after end of treatment are included.
AE = adverse event, NOS = not otherwise specified.

7.7.3 ENABLE

In the ongoing, blinded ENABLE study in severe CHF, a total of 474 (29.4%) of the 1613 patients had one or more SAEs as of the clinical cut-off date. The most frequent (≥ 1%) SAEs were cardiac failure, chest pain, pneumonia, cardiovascular accident, renal failure, unstable angina, syncope, abdominal pain, anemia, dehydration and dyspnea. These SAEs were very similar to those reported in the other studies in CHF patients, were related to the underlying diseases, and were not unexpected in this patient population.

7.8 Premature Discontinuations Due to Adverse Events

Of the 715 patients treated with bosentan, 90 (12.6%) were prematurely discontinued due to adverse experiences (clinical AEs, laboratory abnormalities and ECG abnormalities). In addition, 4 of 416 (1.0%) healthy volunteers and patients exposed to bosentan in the clinical pharmacology program were prematurely withdrawn due to adverse experiences. The most frequent (> 1%) AEs leading to premature withdrawal were abnormal hepatic function, headache, and cardiac failure.

7.8.1 All Placebo-controlled Studies

In the pool of placebo-controlled studies, the incidence of premature withdrawal with bosentan due to AEs was slightly higher than with placebo (11.1% vs 9.4%, respectively). Evaluating the reasons for premature withdrawal by dose revealed that the overall incidence of withdrawal with doses up to 500 mg/day was lower than in the placebo group (4.7% vs 8.5%, respectively, at 250-500 mg/day). Patients prematurely withdrawn due to abnormal hepatic function, anemia, or hypotension were mainly CHF patients treated with the high dose of bosentan (1000-1500 mg/day), and headache was the main reason for premature withdrawal among

bosentan-treated patients treated with 2000 mg/day. No patient receiving bosentan doses of 100 to 500 mg/day was withdrawn for anemia or hypotension.

7.8.2 Studies in Pulmonary Arterial Hypertension

In the pivotal studies in pulmonary arterial hypertension, a greater proportion of patients on placebo than on bosentan were prematurely discontinued because of an AE (10.0% vs 5.5%, respectively, Table 28). Aggravated PHT (7.5%) was the most frequent reason for discontinuation among placebo-treated patients, and abnormal hepatic function (1.8%) among bosentan-treated patients. One patient has been withdrawn from treatment during the extension study (AC-052-353) because of aggravated PHT and was put on epoprostenol therapy.

Table 28 Adverse events leading to withdrawal in placebo-controlled studies in pulmonary arterial hypertension, safety population

(Table T56_12y / 21MAY01)
Protocols: AC-52351 AC-52352

Body system / Adverse event	Bosentan 250 mg/d N=95 No.	%	Bosentan 500 mg/d N=70 No.	%	Bosentan (all) N=165 No.	%	Placebo N=80 No.	%
ALL BODY SYSTEMS								
Total pts with at least one AE	3	3.2%	6	8.6%	9	5.5%	8	10.0%
Total number of AEs	3		9		12		10	
HEPATIC FUNCTION ABNORMAL NOS	-		3	4.3%	3	1.8%	-	
PULMONARY HYPERTENSION NOS AGGRAVATED	2	2.1%	-		2	1.2%	6	7.5%
CARDIAC FAILURE NOS	1	1.1%	1	1.4%	2	1.2%	1	1.3%
CHOLELITHIASIS	-		1	1.4%	1	0.6%	-	
JAUNDICE NOS	-		1	1.4%	1	0.6%	-	
PNEUMONIA NOS	-		1	1.4%	1	0.6%	-	
RENAL FAILURE NOS	-		1	1.4%	1	0.6%	-	
SEPSIS NOS	-		1	1.4%	1	0.6%	-	
SYNCOPE	-		-		-		2	2.5%
RIGHT VENTRICULAR FAILURE	-		-		-		1	1.3%

AE = adverse event, NOS = not otherwise specified.

7.8.3 ENABLE

In the still blinded ENABLE study, 139 (8.6%) patients have been prematurely withdrawn from study treatment because of an AE or lack of treatment benefit. The only reasons for withdrawal that were reported for at least 0.5% of patients were cardiac failure (1.6%), fatigue (0.7%), dyspnea (0.6%), abnormal hepatic function (0.6%), and dizziness (0.5%).

7.9 Clinical Laboratory Test Abnormalities

Laboratory values were transformed where necessary and compared to the Sponsor's reference values (Appendix 14) for determining marked abnormalities.

In placebo-controlled studies, mean changes from baseline (to study end or to the worst laboratory value during the study) and incidences of MLAs were evaluated. For most laboratory

parameters, the differences between bosentan- and placebo-treated patients were small and not clinically relevant. Regardless of the method of analysis, the changes in laboratory parameters that were different between treatment groups, clinically relevant, and associated with bosentan treatment were elevations in liver aminotransferases (ALT and AST) and decreases in RBC parameters. These laboratory abnormalities were also observed in patients with pulmonary arterial hypertension, and are discussed in detail in Sections 8 and 9, respectively.

7.10 Electrocardiography

The treatment-emergent ECG changes presented include all ECG changes, regardless of clinical relevance and including those that were transient, observed during the regular 12-lead ECGs or rhythm strips recorded at the scheduled visits, as well as from any additional ECG recording performed during the conduct of a study.

7.10.1 Treatment-emergent ECG Changes

Overall, the proportion of patients with at least one ECG abnormality in the integrated database of placebo-controlled studies was greater in the placebo group than in the bosentan group (34.4% vs 24.5%, respectively, Table 29).

Table 29 Treatment-emergent ECG findings in the placebo-controlled studies, safety population

(Table T65f / 17MAY01)
Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031

ECG finding	Placebo N=288 No.	%	Bosentan N=677 No.	%
Total pts with at least one ECG finding	99	34.4%	166	24.5%
Total number of ECGs findings	151		240	
ST-T CHANGES	16	5.6%	28	4.1%
ATRIOVENTRICULAR BLOCK FIRST DEGREE	17	5.9%	23	3.4%
INTRA-VENTRICULAR CONDUCTION DEFECT	9	3.1%	22	3.2%
VENTRICULAR EXTRASYSTOLES	13	4.5%	21	3.1%
RIGHT AXIS DEVIATION	11	3.8%	13	1.9%
NON SPECIFIC ST-T CHANGES	5	1.7%	11	1.6%
ATRIAL FLUTTER AND / OR FIBRILLATION	2	0.7%	11	1.6%
LEFT ATRIAL ENLARGEMENT	1	0.3%	11	1.6%
SINUS BRADYCARDIA	8	2.8%	10	1.5%
SINUS TACHYCARDIA	9	3.1%	9	1.3%
EVIDENCE OF MYOCARDIAL INFARCTION	7	2.4%	9	1.3%
RIGHT VENTRICULAR HYPERTROPHY	5	1.7%	9	1.3%
OTHER FINDINGS	1	0.3%	8	1.2%
LEFT ANTERIOR HEMI-BLOCK	2	0.7%	7	1.0%
LEFT AXIS DEVIATION	1	0.3%	7	1.0%
RIGHT ATRIAL ENLARGEMENT	6	2.1%	6	0.9%
SUPRAVENTRICULAR EXTRASYSTOLES	5	1.7%	6	0.9%
BUNDLE BRANCH BLOCK LEFT	6	2.1%	5	0.7%
LEFT VENTRICULAR HYPERTROPHY	9	3.1%	4	0.6%
BILATERAL ATRIAL ENLARGEMENT	1	0.3%	4	0.6%
SINUS RHYTHM	-		4	0.6%
BUNDLE BRANCH BLOCK RIGHT	2	0.7%	3	0.4%
PROLONGED QT	5	1.7%	2	0.3%
PACEMAKER RHYTHM	1	0.3%	2	0.3%
SINUS ARRHYTHMIA	1	0.3%	2	0.3%
HIGH VOLTAGE	2	0.7%	1	0.1%
ATRIOVENTRICULAR BLOCK SECOND DEGREE MOBITZ I	1	0.3%	1	0.1%
LOW VOLTAGE	1	0.3%	1	0.1%
LEFT POSTERIOR HEMI-BLOCK	2	0.7%	-	
ATRIOVENTRICULAR BLOCK COMPLETE	1	0.3%	-	
BUNDLE BRANCH BLOCK BILATERAL	1	0.3%	-	

ECG = electrocardiographic.

Neither the overall incidence of treatment-emergent ECG changes nor the frequency of any single ECG change appeared to be dose related. No relevant ECG changes were consistently more frequent in bosentan-treated patients than in those on placebo across the four dose groups.

Treatment-emergent ECG abnormalities were reported as AEs when investigators assessed them as clinically relevant. Atrial fibrillation (reported in 1.5% and 1.4% of bosentan- and placebo-treated patients, respectively) was the only ECG change reported as an AE in at least 1% of bosentan-treated patients. The low frequency and pattern of ECG changes reported as AEs suggest that they were isolated events and not related to bosentan treatment.

Many patients with pulmonary arterial hypertension had ECG abnormalities at baseline, but no clinically relevant treatment-emergent ECG changes were observed in either of the two pivotal placebo-controlled studies (AC-052-351 and AC-052-352, Appendix 15).

7.10.2 Mean Changes in Heart Rate, PQ-, QRS-, and QT-Intervals

In the placebo-controlled studies, there was no evidence for any clinically relevant mean changes from baseline to study end in heart rate, PQ-, QRS-, or QT-intervals (Table 30). Across the four

dose groups, bosentan had no clinically relevant effects on heart rate, PQ-, QRS-, and QT-intervals. The absence of any dose effect of bosentan on ECG parameters was also seen in the dose-finding study in systemic hypertension (NC15020).

Table 30 Mean changes from baseline to study end in heart rate, PQ-, QRS-, and QT-intervals in placebo-controlled studies, safety population

(Table T61f / 17MAY01)
Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031

		Placebo N=288			Bosentan N=677		
Parameter	Unit	N	BL	Change	N	BL	Change
HEART RATE	(bpm)	243	78.1	0.4	539	76.1	-1.0
PQ (PR)	(ms)	206	172.5	3.5	478	172.3	0.4
QRS	(ms)	141	94.1	1.8	379	93.4	-0.2
QT	(ms)	238	382.0	1.7	536	387.9	0.2

As with the overall population, bosentan treatment had no clinically relevant effect on heart rate, PQ-, QRS-, and QT-intervals in patients with CHF or in those with systemic hypertension. Similarly, no relevant changes in heart rate, PQ-, QRS-, or QT-intervals were observed in either treatment group in the two pivotal studies in pulmonary arterial hypertension (AC-052-351 and AC-052-352, Appendix 16). Mean (± SD) changes in heart rate with bosentan were −1.2 ± 13.0 bpm and −1.8 ± 11.6 bpm in the two studies, respectively, and with placebo were 3.2 ± 7.0 bpm and 1.8 ± 11.5 bpm, respectively.

The absence of any clinically relevant changes in ECG parameters is consistent with the preclinical electrophysiological findings indicating that bosentan had no effect on the cardiac conduction system [13].

7.11 Vital Signs

Mean changes in vital sign parameters were evaluated by calculating the changes from baseline to the last measurement during active treatment.

7.11.1 Mean Changes in Vital Signs

In the pool of placebo-controlled studies, bosentan administration was not associated with clinically relevant changes in pulse rate at any dose (Table 31), reiterating the results of ECG assessments. Small decreases in systolic and diastolic blood pressures were observed among bosentan-treated patients, but the differences between bosentan- and placebo-treated patients were small. The decreases in blood pressures did not appear to be dose related in the combined placebo-controlled studies, but tended to be dose dependent in the dose-finding hypertension study (NC15020).

Table 31 Vital signs: Mean change from baseline to end of study in placebo-controlled studies, safety population

(Table T71f / 17MAY01)
Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031

		Placebo N=288			Bosentan N=677		
Parameter	Unit	N	BL	Change	N	BL	Change
SYS. BLOOD PRESSURE	(mmHg)	282	126.4	-2.4	662	130.3	-3.1
DIAS. BLOOD PRESSURE	(mmHg)	282	78.5	-0.4	662	81.3	-3.0
HEART RATE	(bpm)	282	78.4	0.3	663	77.0	0.2
WEIGHT	(kg)	201	77.2	0.4	396	75.2	0.3

No clinically relevant effects on heart rate or blood pressures were observed in the clinical pharmacology studies with oral bosentan at doses up to 1000 mg.

Patients with pulmonary arterial hypertension in the two pivotal placebo-controlled studies exhibited small mean decreases in systolic and diastolic blood pressures with no associated meaningful change in heart rate (Table 32).

Table 32 Vital signs: Mean change from baseline to end of study in placebo-controlled studies in pulmonary arterial hypertension, safety population

	Bosentan dose (mg b.i.d.)			
	125 (N = 95)	250 (N = 70)	All doses (N = 165)	Placebo (N =80)
Systolic BP (mmHg)				
Baseline	119.4 ± 20.8	117.1 ± 15.9	118.4 ± 18.9	121.6 ± 19.3
Change to study end	−2.9 ± 19.0	−6.0 ± 14.9	−4.2 ± 17.4	−3.8 ± 16.2
Diastolic BP (mmHg)				
Baseline	73.8 ± 11.3	73.8 ± 12.4	73.8 ± 11.7	75.2 ± 11.0
Change to study end	−3.4 ± 12.8	−3.0 ± 12.1	−3.3 ± 12.5	0.7 ± 10.4
Pulse Rate (bpm)				
Baseline	82.3 ± 14.5	83.8 ± 13.0	82.9 ± 13.8	81.8 ± 12.1
Change to study end	−2.2 ± 12.8	0.7 ± 14.5	−1.0 ± 13.6	3.3 ± 13.5

Note: Values are mean ± standard deviation. Includes Protocols AC-052-351 and AC-052-352.
BP = blood pressure, bpm = beats per minute.

7.11.2 Incidence of Hypotension

The incidence of hypotension (symptomatic or asymptomatic), defined as a decrease in systolic blood pressure to less than 80 mmHg at any time during the trials, whether or not reported as an AE, was evaluated across all placebo-controlled therapeutic studies. The different confounding factors of dose, indication, and planned treatment duration were taken into consideration.

Based on this approach, no evidence was found that treatment with bosentan was associated with an increased incidence of hypotension. In fact, in most cases the incidence was higher among placebo-treated patients. This observation is supported by the reports of hypotension and postural hypotension as AEs (see Section 7.5.1). The overall incidence of hypotension reported as an AE among bosentan- and placebo-treated patients was 6.8% and 7.6%, respectively, and the overall incidence of postural hypotension was 1.9% and 4.9%, respectively. Similarly, in the pool of placebo-controlled studies the incidences of dizziness and syncope were greater in the placebo group than in the bosentan group.

In the pivotal studies in patients with pulmonary arterial hypertension, there were no reports of postural hypotension, and the incidences of dizziness and syncope were slightly greater on placebo. The incidence of hypotension was greater in patients on bosentan than in those on placebo (6.7% with bosentan and 3.8% with placebo), but all of the patients who experienced hypotension remained on bosentan treatment. Very high concentrations of bosentan as were obtained with the i.v. infusion in the exploratory study in pulmonary arterial hypertension (BD14884) could be associated with an increased risk of hypotension (see Appendix 1).

7.12 Drug–demographic, Drug–disease, and Drug-drug Interactions

The evaluation of the safety and the tolerability of bosentan in subgroups of patients was performed on the pool of bosentan and corresponding placebo patients enrolled in the eight placebo-controlled trials in the therapeutic program. Safety parameters analyzed in the pooled analyses were the overall incidence of AEs, the incidences of the five most frequent AEs identified as related to bosentan treatment (abnormal hepatic function, anemia, flushing, headache, leg edema), the overall incidence of SAEs, and the incidence of MLAs shown to be related to bosentan treatment (markedly low hemoglobin and ALT and/or AST > 3 × ULN).

The small number of patients and events in certain groups as well as the confounding covariables requires that caution be used in the interpretation of particular aspects of the subgroup analyses.

Overall, there were few differences in any of the safety parameters evaluated between any of the subpopulations analyzed, and the observed differences were either of minor clinical significance or were of a pattern expected for that particular subpopulation (e.g., as demonstrated by similar differences among patients treated with placebo). The only exception was a higher incidence of elevated liver aminotransferases found when bosentan was given concomitantly with glibenclamide (glyburide), which is further discussed in Section 8. Accordingly, these safety subpopulation analyses support the recommendation that no dose adjustment of bosentan is needed for any specific subpopulation.

8 EFFECTS OF BOSENTAN ON HEPATIC ENZYMES

The mechanism that might be associated with the bosentan-related increase in liver aminotransferases has been investigated in both *in vitro* and *in vivo* experiments and by a careful examination of the clinical data. This Section first summarizes the preclinical data that relate to the occurrence of this laboratory abnormality in bosentan-treated patients and its potential mechanisms (see also Section 3). Second, a full description of the clinical picture drawn from the entire database (including still blinded trials) is provided, with an estimate of the risk that might be associated with the elevated liver aminotransferases.

8.1 Preclinical Data on Bosentan-induced Changes in Liver

8.1.1 Toxicology Studies

In toxicology studies, high dosages of bosentan caused mild signs of cholestasis. While an increase in alkaline phosphatase was a consistent observation in all studies, a transient increase in ALT was observed only in the 4-week dog study. No histological evidence was found for hepatoxicity in marmosets, rats, or dogs given daily bosentan dosages in 4-week, 6-month and 12-month studies.

Increased liver weights were observed in most studies, particularly at the higher dosages. Histological features included minimal to slight bile duct proliferation and slight increase in single cell necrosis (only in the 4-week dog study), low-grade periacinar hypertrophy, and mild signs of cholestasis. No associated signs of centrolobular necrosis were observed.

8.1.2 Mechanistic Studies

Bosentan and its metabolites are eliminated almost entirely in bile. Mechanistic studies show that a likely mechanism for the liver enzyme changes with bosentan is a concentration-dependent competition by bosentan and its metabolites with the biliary elimination of bile salts, resulting in a retention of bile salts and a secondary increase in serum aminotransferases. Accordingly, serum bile salts were markedly increased in a dose-dependent manner in dogs. *In vitro* studies demonstrated that bosentan inhibited taurocholate transport across rat bile salt export system) in a dose-dependent manner. Furthermore, a potentiation of the increase in plasma bile salt concentration occurred in rats when bosentan was given concomitantly with glibenclamide (glyburide), another compound that competes with bile salt elimination. In contrast, there is no evidence for the formation of reactive metabolites, an immuno-allergic mechanism, mitochondrial toxicity, or direct hepatotoxicity of bosentan or its metabolites.

8.2 Clinical Data on Bosentan-induced Increases in Liver Aminotransferases

In this report, clinically meaningful increases in liver aminotransferases were defined as ALT and/or AST > 3 × ULN. All values are presented in Système International units, and sponsor-defined reference ranges are presented in Appendix 14. 'Transient' was used to describe an increase in ALT and/or AST > 3 × ULN that returned to either baseline levels or not more than 2 × ULN while treatment with study medication continued. Possibly associated symptoms were

based on the report of a relevant AE within ± 2 weeks of the elevated liver aminotransferases. AEs considered relevant were abdominal pain, nausea, vomiting, jaundice, and pyrexia.

8.2.1 Overall Incidence and Clinical Characteristics

In the pool of placebo-controlled trials, treatment with bosentan was associated with an increased incidence in elevated ALT and/or AST to > 3 × ULN in 74/658 patients who had measurements of ALT or AST (11.2% vs 1.8% among placebo-treated patients, Table 33 and Appendix 17). This increase in liver aminotransferases was observed in all populations studied (Appendix 17). In some cases these increases were reported by the investigator as AEs, and in the pool of completed placebo-controlled trials, 'abnormal hepatic function' was reported for 5.9% of bosentan-treated patients vs 2.1% of placebo-treated patients.

Dose relationship. The elevation in liver aminotransferases associated with bosentan treatment was dose related. This conclusion is based on the following observations:

– The overall incidence by dose (Table 33).

– The incidence in the dose-finding study in hypertensive patients (NC15020, Table 33).

– The greater proportion of severe cases at higher bosentan doses (Table 35)

– The observed decrease or complete resolution of the elevated liver aminotransferases in some patients (CHF and pulmonary arterial hypertension studies) when the bosentan dose is decreased (an example of such a case is provided in Appendix 18, Patient 208 20007).

Table 33 Incidence of ALT and/or AST > 3 × ULN among bosentan-treated patients in placebo-controlled studies, safety population

Indication	Bosentan dose (mg/day) 100	250/500	1000/1500	2000	All bosentan doses
PAH	—	21/165 (12.7%)	—	0/4 (0%)	21/169 (12.4%)
CHF	—	—	37/234 (15.8%)	2/48 (4.2%)	39/282 (13.8%)
HTN	1/48 (2.1%)	2/46 (4.3%)	5/44 (11.4%)	5/50 (10.0%)	13/188 (6.9%)
SAH	—	—	1/19 (5.3%)	—	1/19 (5.3%)
All patients	1/48 (2.1%)	23/211 (10.9%)	43/297 (14.5%)	7/102 (6.9%)	74/658 (11.2%)

Note: Values are the number of cases/total number assessed (%) for each dose and indication group. More detailed information, including the incidence among placebo-treated patients, can be found in Appendix 17
The sponsor's upper limit of the normal range was 30 U/l for ALT and 25 U/l for AST.
ALT = alanine aminotransferase, AST = aspartate aminotransferase, CHF = chronic heart failure, HTN = hypertension, PAH = pulmonary arterial hypertension, SAH = subarachnoid hemorrhage, ULN = upper limit of normal.

Severity. The magnitude of the increase in liver aminotransferases observed in the 74 bosentan-treated patients was categorized according to the maximal increase during the trial (Table 34).

Table 34 Magnitude of the increase in ALT and/or AST > 3 × ULN among bosentan-treated patients (N = 658) in placebo-controlled studies

	ALT and/or AST values > 3 and < 5 × ULN	≥ 5 and < 8 × ULN	≥ 8 × ULN	All cases > 3 × ULN
Patients with elevated LFTs	29 (4.4%)	19 (2.9%)	26 (3.9%)	74 (11.2%)

Note: Values are the number of cases (% of all patients assessed, N = 658) for each magnitude category.
The sponsor's upper limit of the normal range was 30 U/l for ALT and 25 U/l for AST.
ALT = alanine aminotransferase, AST = aspartate aminotransferase, LFT = liver function test, ULN = upper limit of normal.

In the pivotal studies in pulmonary arterial hypertension (AC-052-351 and AC-052-352), 21 (12.7%) of the 165 patients on bosentan had an increase in liver aminotransferases to > 3 × ULN. When the magnitudes of these increases were categorized by dose (Table 35), it was apparent that the magnitude of the increase was dose related, which further supports the dose-related nature of the abnormality. A greater proportion of patients on bosentan 250 mg b.i.d. had an increase to ≥ 8 × ULN than did those on 125 mg b.i.d. Moreover, for three of the patients treated with the 250 mg b.i.d. dose, treatment had to be discontinued because of elevated liver aminotransferases (all were ≥ 8 × ULN), which was not necessary for any of the patients on the lower dose of the compound. Thus, not only was the overall incidence dose related, but also the severity.

Table 35 Magnitude of the increase in ALT and/or AST > 3 × ULN among bosentan-treated patients with pulmonary arterial hypertension (AC-052-351 and AC-052-352)

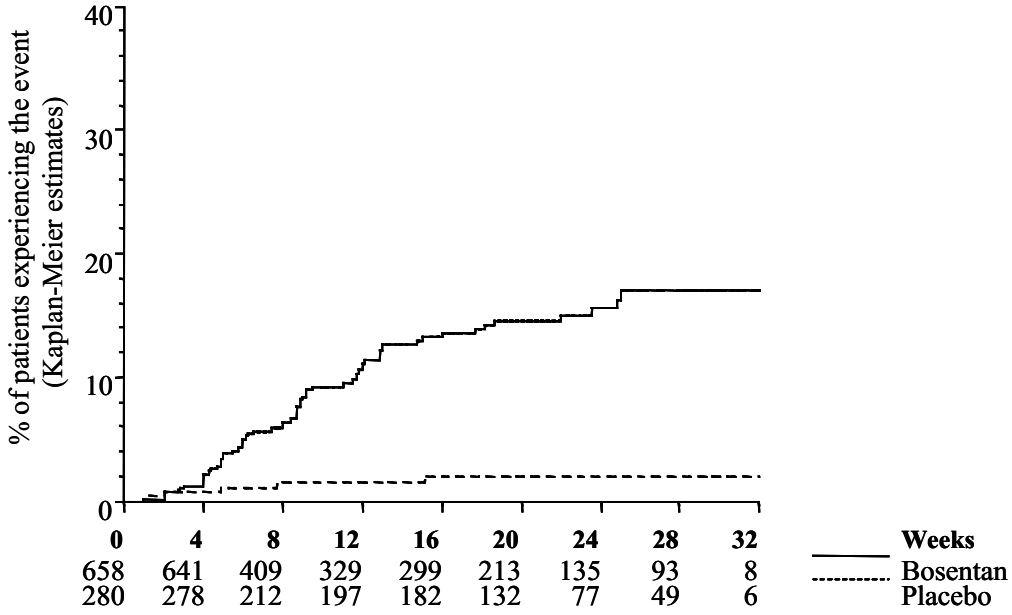
	ALT and/or AST values			
Bosentan Treatment	> 3 and < 5 × ULN	≥ 5 and < 8 × ULN	≥ 8 × ULN	All cases > 3 × ULN
125 mg b.i.d. (n = 95)	7 (7.4%)	2 (2.1%)	2 (2.1%)	11 (11.6%)
250 mg b.i.d. (n = 70)	4 (5.7%)	1 (1.4%)	5 (7.1%)	10 (14.3%)
Both doses (n = 165)	11 (6.7%)	3 (1.8%)	7 (4.2%)	21 (12.7%)

Note: Values are the number of cases (% of patients in dose group) for each dose group.
The sponsor's upper limit of the normal range was 30 U/l for ALT and 25 U/l for AST.
ALT = alanine aminotransferase, AST = aspartate aminotransferase, ULN = upper limit of normal.

Time to occurrence. In most cases, the increase in liver aminotransferases developed over several weeks of treatment. In about 90% of cases (67 of the 74 cases in placebo-controlled studies and 18 of the 20 cases in Study AC-052-352 in pulmonary arterial hypertension), the first elevation in liver aminotransferases (> 3 × ULN) was detected during the first 16 weeks of bosentan treatment (Figure 26 and Figure 27). The two cases in Study AC-052-352 that occurred after Week 16 were a transient increase to > 3 × ULN and an increase to < 4 × ULN with the 125 mg b.i.d. dose of bosentan.

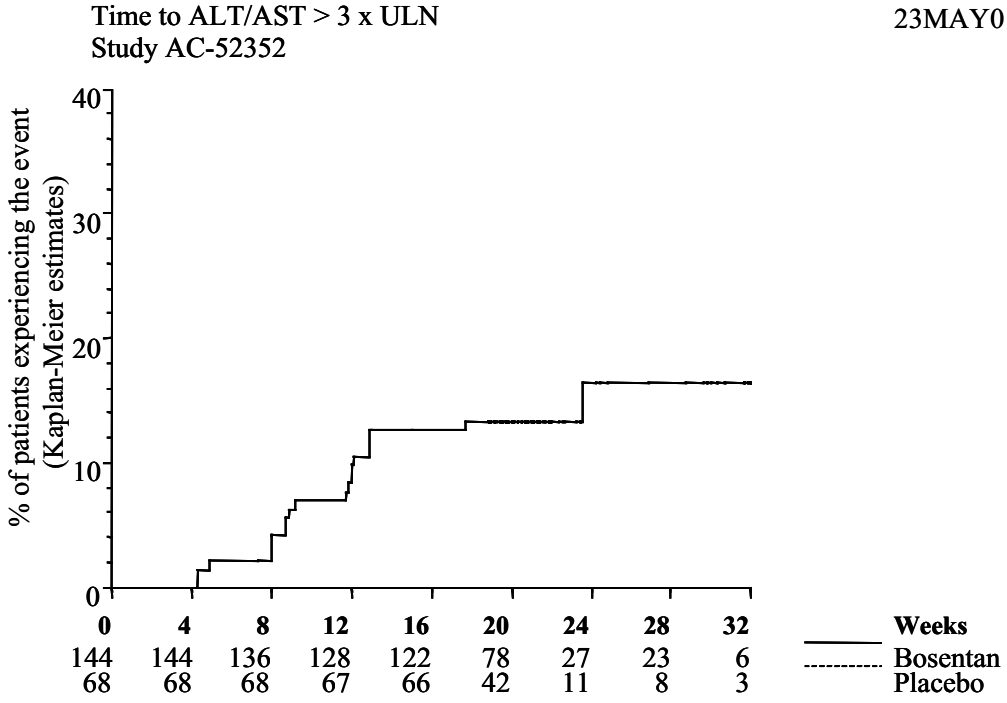
Figure 26 **Kaplan-Meier estimates of time to first appearance of ALT and/or AST > 3 × ULN in placebo-controlled studies, safety population**

Time to ALT/AST > 3 x ULN 23MAY01
Studies: AC-52351 AC-52352 BC- 15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031



Patients not experiencing the event are censored 28 days after the end of treatment.

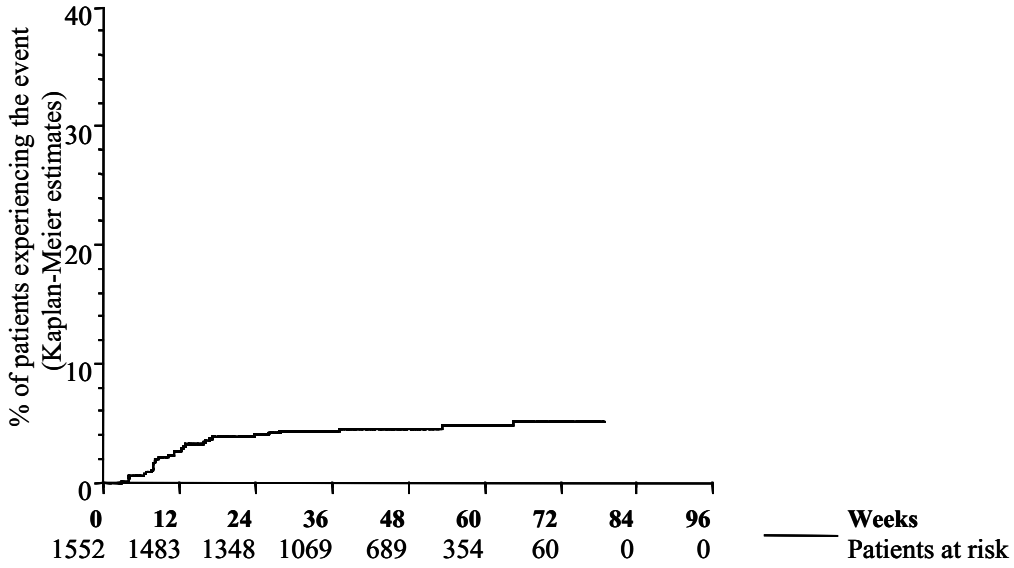
Figure 27 **Kaplan-Meier estimates of time to first appearance of ALT and/or AST > 3 × ULN in Study AC-052-352 in pulmonary arterial hypertension, safety population**



Patients not experiencing the event are censored 28 days after the end of treatment.

Similar observations were made in the ongoing, still blinded ENABLE study (N = 1613, Figure 28). In these trials, 69 (4.3%) of the 1613 patients treated for a mean (± SD) of 321 ± 119 days had an increase in liver aminotransferases to > 3 × ULN (clinical cut-off on 31 December 2000).

Figure 28 **Kaplan-Meier estimates of time to first appearance of ALT and/or AST > 3 × ULN in the ongoing, blinded ENABLE study in chronic heart failure**



Resolution. In most cases the increase in liver aminotransferases developed over several weeks of treatment, and in all cases, stopping treatment resulted in rapid and complete reversal of the elevated liver aminotransferases (an example is provided in Appendix 19, Patient 106 10008). Treatment was stopped because of elevated liver aminotransferases for 28 (4.1%) of the 677 patients in placebo-controlled studies and for 5 patients in the open-label studies, and there was no evidence for continued liver injury after treatment cessation. Similar observations were made in the ongoing, blinded ENABLE study. In these trials, treatment was stopped or interrupted because of an increase in liver aminotransferases in 25 of the 1613 patients (i.e., 1.5% of the population or 3.0% of bosentan-treated patients, if all cases occurred in this treatment group). In 14 of these patients, the low dose of study treatment (bosentan or placebo) was reintroduced after the increases were reversed. In these 14 patients, 5 had no new increase in liver aminotransferases, 5 had a transient increase that returned to baseline levels while treatment with the study drug continued, and 4 patients were permanently discontinued because of a recurrent increase in liver aminotransferases. The remaining 11 patients were permanently discontinued (not necessarily due to elevated liver aminotransferases), and reintroduction of study treatment was not attempted. Treatment cessation or interruption in ENABLE resulted in a rapid decline in the liver aminotransferases to baseline levels or to ≤ 2 × ULN within a few days to 6 weeks (depending on the timing of the test) in all but six patients. For three of the six patients, no further values were available, and in the other three patients, other reasons (hepatitis C, hepatitis A, and ovarian neoplasm) for the increase in liver aminotransferases were suspected.

Transient cases. Occasionally, the increase in liver aminotransferases was transient, and a reversal of the abnormality back to baseline levels or to ≤ 2 × ULN occurred either during continued treatment with the target dose of bosentan or after dose reduction (examples are provided in Appendix 18, Patient 208 20007 and Appendix 20, Patient 109 10065, respectively). The frequency of this observation in patients with pulmonary arterial hypertension treated with bosentan doses of 125 mg or 250 mg b.i.d. is described in Table 36. Transient cases (reversal of the increase in liver aminotransferases while continuing treatment with the target dose or with a reduced dose) were more common among patients on the 125 mg b.i.d. dose than among those on the 250 mg b.i.d. dose. Furthermore, patients on 125 mg b.i.d. were more likely than those on the higher dose to have remained on the target dose rather than to have had their dose reduced.

Table 36 Transient increases in ALT and/or AST to > 3 × ULN among bosentan-treated patients with pulmonary arterial hypertension (AC-052-351 and AC-052-352)

	Transient elevations during continued treatment		
Bosentan dose group	Stable dosing	Dose reduction	All cases
125 mg b.i.d. (n = 95)	7/11 (64%)	1/11 (9%)	8/11 (73%)
250 mg b.i.d. (n = 70)	3/10 (30%)	2/10 (20%)	5/10 (50%)
Both doses (n = 165)	10/21 (48%)	3/21 (14%)	13/21 (62%)

Note: Values are number of transient cases/number of cases in that dose group.
The sponsor's upper limit of the normal range was 30 U/l for ALT and 25 U/l for AST.
ALT = alanine aminotransferase, AST = aspartate aminotransferase, ULN = upper limit of normal.

Similar transient cases occurred in the ongoing, blinded ENABLE study, if one assumes that all cases of elevated liver aminotransferases were in the bosentan group (69 cases in approximately 807 patients randomized to bosentan, or 8.6%, Table 37). More than half of the 69 patients (n = 38) who had an increase in liver aminotransferases (> 3 × ULN) had a transient increase during continued treatment with study drug (bosentan or placebo).

Table 37 Increases in ALT and/or AST to ≥ 3 × ULN in the ongoing, blinded ENABLE study in chronic heart failure

	ALT and/or AST value			
	> 3 and < 5 × ULN	≥ 5 and < 8 × ULN	≥ 8 × ULN	All cases > 3 × ULN
Total*	31 (3.8%)	15 (1.9%)	23 (2.9%)	69 (8.6%)

*Total percentages are based on the assumption that all cases occurred in the approximately 807 patients treated with bosentan and none in the approximately 806 placebo-treated patients (randomization 1:1).

Confounding factors. The incidence of increased liver aminotransferases (> 3 × ULN) during treatment among bosentan-treated patients was evaluated based on the presence or absence of ALT/AST or alkaline phosphatase values above the ULN at baseline. Based on this approach, the incidence of increases in liver aminotransferases during treatment was not affected by the presence of elevated alkaline phosphatase at baseline. In contrast, the incidence of increased liver aminotransferases was greater among patients with elevated liver aminotransferases at baseline than among those with normal values at baseline (16.54% vs 9.98%, respectively). Similar observations were made in the ongoing ENABLE study.

There was no evidence of a relationship between elevated liver aminotransferases and demographic factors such as age, gender, race or weight. Screening for the potential role of concomitant medications revealed a possible pharmacodynamic drug interaction between glibenclamide (glyburide) and bosentan. Thus, the incidence of increased liver aminotransferases among patients who took the combination of bosentan and glibenclamide compared to those who did not was 27.5% vs 13.3%, respectively (NC15462, REACH-1 trial). This may be explained by the synergistic effect of the two drugs, when given together, on the Bsep as described in Sections 3 and 4.2.

8.2.2 Associated Symptoms

Typically, the increases in liver aminotransferases were asymptomatic. Temporally associated (though not necessarily causally related) symptoms known to occur in patients with liver injury were reported for 9 of the 74 bosentan-treated patients in the placebo-controlled studies who had elevated liver aminotransferases. Symptoms included abdominal pain (2 patients), nausea/ vomiting (3), and fever (4). Elevated bilirubin (> 3 × ULN) was observed in one patient. One case of jaundice was reported in the open-label extension study in patients with severe CHF (NC15464B). In all cases, symptoms rapidly and completely disappeared when treatment was stopped. One patient (18165/6343, Study NC15462) had pruritic rash concomitant with the increase in liver aminotransferases, with no increase in bilirubin or alkaline phosphatase. The investigator indicated that the rash was secondary to treatment with atorvastatin (Lipitor®, Pfizer, Inc.). This conclusion was based on the following observations: the rash resolved when Lipitor was stopped, it re-appeared when Lipitor was re-introduced, and it resolved again when Lipitor was discontinued, all during continued bosentan treatment.

Similar observations were made in the ongoing, blinded ENABLE study. Here too, the increases in liver aminotransferases were typically asymptomatic. Associated symptoms were reported in 11 of the 69 patients with elevated liver aminotransferases in these studies. Symptoms included abdominal pain (6 patients), nausea/vomiting (4), fever (2), and jaundice (1). Two additional patients had an increase in liver aminotransferases to > 3 × ULN and a marked increase in bilirubin to > 3 × ULN, but the reasons for these increases were acute hepatitis A and end stage heart failure (Patients 50427 and 41702, respectively, see narratives in Section 8.2.3).

To date, there have been no cases of liver failure, liver transplant, or death due to liver failure among bosentan-treated patients.

8.2.3 Risk Assessment

The Zimmerman criteria [15] for risk assessment in cases of elevated liver aminotransferases (ALT > 3 × ULN and total bilirubin > 3 × ULN, with little change in alkaline phosphatase, i.e., < 2 × ULN) were used to evaluate the risk of liver failure with bosentan treatment. Two bosentan-treated patients in the integrated safety database and three in the still blinded ENABLE study) had both elevated liver aminotransferases (> 3 × ULN) and elevated bilirubin (total bilirubin > 3 × ULN). However, two of the ENABLE patients had clear evidence for other reasons for the elevated liver function tests (hepatitis A in Patient 50427 and end-stage heart failure in Patient 41702; see narratives below). The other three patients (one of which is still blinded as to treatment [ENABLE]) had an increase in alkaline phosphatase (2–3 × ULN) in addition to the elevated liver aminotransferases. Nevertheless, for these three patients, a relationship to treatment could not be ruled out with certainty. Although none of these patients strictly met the Zimmerman criteria (as all had an increase in alkaline phosphatase), based on a conservative approach, the risk of having both increased liver aminotransferases and elevated bilirubin (each > 3 × ULN) on bosentan treatment was calculated to be 1/500. This is based on 3 cases in the 1522 patients exposed to bosentan in the clinical trials (715 patients in placebo-controlled and open-label trials and approximately 807 patients on bosentan in ENABLE). As it is estimated that 10% of patients with both ALT and bilirubin > 3 × ULN will develop liver failure, the risk of liver failure with bosentan is estimated at 1/5000 patients.

The five cases with marked increase in bilirubin to > 3 × ULN are described below.

Patient 106/10008, Study AC-052-352. This 53-year-old black female had pulmonary arterial hypertension due to mixed connective tissue disease. She had a history of hepatic manifestations of lupus erythematosus (confirmed by liver biopsy), and treatment with hydroxychloroquine for lupus was stopped shortly before the trial. On Day 65 of treatment with bosentan 250 mg b.i.d., her liver aminotransferases increased to > 3 × ULN. Despite dose reduction, the liver aminotransferases continued to increase, reaching the highest level on Day 143. On that day, the ALT was 420 U/l (ULN = 30 U/l), alkaline phosphatase was 253 U/l (ULN = 95 U/l) and total bilirubin was 52.9 µmol/l (ULN = 17.1 µmol/l). There was no increase in eosinophils. The patient was asymptomatic during the entire period, and jaundice or other symptoms were not reported as AEs. Stopping treatment resulted in rapid and complete reversal of the elevated liver function tests (see Appendix 19).

Patient 20060/00841, Study NC15464. This 81-year-old white male with ischemic heart disease and NYHA class IV heart failure was hospitalized on Day 560 of the open-label extension trial (NC 15464B) with a 1-week history of persistent vomiting, lower abdominal pain, jaundice, and weight loss. Physical examination revealed jaundice, distended abdomen, and a palpable, tender epigastric mass. Laboratory tests revealed an increase in ALT, alkaline phosphatase and total bilirubin. Maximal levels were 392 U/l ALT (ULN = 35 U/l), 586 U/l alkaline phosphatase (ULN = 300 U/l), and 91 µmol/l total bilirubin (ULN = 15 µmol/l). Abdominal ultrasound demonstrated a normal gall bladder surrounded by a small quantity of fluid. CT scan did not show an epigastric mass or biliary dilatation; some features of the scan were compatible with a settling episode of pancreatitis. Pleural effusion was observed on chest x-ray. Endoscopy was essentially normal, except for slight inflammation of the second part of the duodenum. Biopsies

of the gastric mucosa demonstrated chemical (reactive) gastritis. No clear diagnosis was made for this episode of vomiting, abdominal pain, and jaundice. Bosentan treatment was stopped 9 days after the start of the symptoms. The patient recovered spontaneously and quickly, and all liver tests returned to baseline levels.

The history of this event is compatible with cholelithiasis secondary to a gallstone and inflammation, leading to a distended gallbladder (epigastric mass) and jaundice. Subsequently, the cholelithiasis may have resolved and left no remaining signs, apart from the inflammation and resolving pancreatitis found on ultrasound and CT scan. An inflammatory/gallstone episode is more probable than an adverse drug reaction for two additional reasons:

1. The patient was treated for 190 days with bosentan 500 mg b.i.d. in the preceding placebo-controlled study (NC14562) with no evidence for an increase in liver aminotransferases, and prior to the current episode, the patient had been successfully exposed to bosentan 125 mg b.i.d. for an additional 560 days.

2. One year before starting treatment with bosentan, the patient experienced an episode of epigastric pain and increases in ALT (130 U/l, ULN = 40 U/l) and AP (904 U/l, ULN = 300 U/l); no conclusive diagnosis was made.

These observations indicate that a study drug-event relationship is unlikely. The patient was reviewed on Day 816 and was doing well.

Patient 50511, ENABLE. This 82-year-old white female with severe CHF (NYHA class IIIb and ejection fraction 27%) was found to have an increase in liver aminotransferases > 3 × ULN on Day 119 of treatment (bosentan 125 mg b.i.d. or placebo). On Day 174, she was hospitalized because of abdominal pain, weakness, nausea and jaundice. Her liver function test values were 183 U/l ALT (ULN = 30 U/l), 341 U/l AST (ULN = 25 U/l), 292 U/l alkaline phosphatase (ULN = 95 U/l) and 115.4 µmol/l total bilirubin (ULN = 17.1 µmol/l; conjugated bilirubin was 48.2 µmol/l). Eosinophil counts remained unchanged. Eight days after stopping treatment, all liver tests were at 50% of their highest levels, and complete recovery was achieved within 18 days (see Appendix 21). Follow-up information on Days 218 and 434 showed normal liver function tests with no evidence for liver disease.

Patient 41702, ENABLE. This 64-year-old white male with severe CHF (NYHA class IIIb and ejection fraction 17%) was hospitalized on Day 94 with worsening heart failure that resolved under treatment. On Day 164, pulmonary hypertension, mitral valve insufficiency and severe dilated cardiomyopathy were identified during elective cardiac catheterization. The patient was hospitalized on Day 237 with severe cerebrovascular accident and treated with acenocoumarol and calcium acetylsalicylate. During his hospital stay, he developed severe left and right-sided heart failure that did not respond to inotropic therapy. Study medication and the majority of the concomitant medications were discontinued on Day 260 because of worsening heart failure and morphine was initiated. Transaminase levels were within normal limits before hospitalization, but ALT increased to between 3 and 4 × ULN on Day 261 (ALT 93 U/l, AST 66 U/l); alkaline phosphatase increased to 141 U/l, and bilirubin increased from 27.2 to 55.3 µmol/l. The

investigator considered that the increases in liver tests were all due to low cardiac output with liver ischemia and congestion. The patient died from progressive heart failure on Day 262.

Patient 50427, ENABLE. This 65-year-old white female with severe CHF (NYHA class IV and ejection fraction 33%) had an increase in liver aminotransferases on Day 59 (ALT 112 U/l, AST 55 U/l) that remained below 5 × ULN up to Day 162. On Day 204, aminotransferases were found to be > 8 × ULN (ALT 382 U/l, AST 211 U/l). Study medication was permanently discontinued on that day. A diagnosis of viral hepatitis A infection was made. Aminotransferases were still above 8 × ULN on Day 243, while bilirubin, which was previously within normal limits, increased to 374.5 µmol/l. Transaminase levels fluctuated above the normal range thereafter until reaching near normal by Week 53. Eosinophil count remained unchanged throughout the event. On Day 465, transaminase levels were again found to be > 3 × ULN (ALT 101 U/l, AST 77 U/l). The investigator considered the liver test abnormalities to be due to the viral hepatitis A infection.

8.2.4 Type of Liver Injury and Possible Mechanisms

The type of liver injury induced by bosentan was classified according to the recommendations of the Council for International Organizations of Medical Science (CIOMS) [16]. In this classification scheme, the liver injury is categorized according to the ratio of concomitant changes in ALT and alkaline phosphatase, which is based on the following formula:

ALT level/its ULN ÷ alkaline phosphatase level/its ULN

In these studies, the ULNs for ALT and alkaline phosphatase were 30 U/l and 95 U/l, respectively. The three categories of liver injury are:

Cholestatic injury:	ratio ≤ 2
Hepatocellular injury:	ratio ≥ 5
Mixed (hepatocellular / cholestatic) injury:	ratio > 2 to < 5

Based on this classification scheme, the 74 cases of increased liver aminotransferases in the placebo-controlled studies were categorized as shown in Table 38. Similar observations were made among 67 of the cases in the ongoing, blinded ENABLE study (for 2 of the 69 cases, concomitant alkaline phosphatase levels were not available).

Table 38 Liver injury classifications in bosentan-treated patients with elevated ALT and/or AST > 3 × ULN in the placebo-controlled and ENABLE studies

	Placebo-controlled studies (N = 658)	ENABLE (N = 807*)
Total number of cases	74 (100%)	67 (100%)
Cholestatic injury	7 (9.5%)	3 (4.5%)
Hepatocellular injury	34 (46.0%)	25 (37.3%)
Mixed (hepatocellular / cholestatic) injury	33 (44.5%)	39 (58.2%)

Note: Values are numbers of cases within the injury class/total number of cases of elevated liver aminotransferases in the database (i.e., 74 in placebo-controlled studies and 67 in ENABLE).

* Treatment still blinded (approximately 807 of 1613 are randomized to bosentan).

The mechanism responsible for the increase in liver aminotransferases has not been elucidated.

Based on preclinical observations, accumulation of bile acids in hepatocytes due to competitive inhibition of the Bsep could play a role in the increases in liver aminotransferases observed with bosentan. Bile salt accumulation in hepatocytes could lead to reversible liver damage (see Section 3). This mechanism has been shown *in vitro* to be concentration related. Further support for this mechanism comes from the observation that co-administration of bosentan and the oral anti-diabetic drug glibenclamide (glyburide) was associated with an increased incidence of elevated liver aminotransferases. Glibenclamide is known to interfere with the hepatobiliary excretion of bile salts.

Direct hepatotoxic effects of bosentan cannot be ruled out. However, neither bosentan nor its major metabolites exhibit cytotoxicity in animals (*in vivo*) or in human hepatocytes (*in vitro*), even at doses and concentrations that were much greater than those clinically relevant. Additionally, no evidence for mitochondrial toxicity has been observed.

Finally, there has been no evidence for immuno-allergic liver injury. This conclusion is based on both the clinical characteristics of the elevations in aminotransferases and the absence of an accompanying increase in eosinophils. The clinical picture consists of a dose relationship, a gradual development of the increase in liver enzymes with a rapid and complete resolution of the findings, and the transient nature of the abnormality while continuing bosentan treatment in about 50% of cases. All of these characteristics are clearly not features of an immuno-allergic reaction.

In three patients (2001, 6331 and 8082) in Study NC15462 who had a large increase in ALT levels to > 15 × ULN (492, 1980 and 1217 U/l, respectively), additional tests were performed to detect the presence of selected auto-antibodies and IgE as signals of an immune response. The results of these tests were negative for all three patients, and treatment cessation was associated

with rapid and complete reversal of the increase in liver aminotransferases (see Appendix 22). In addition, none of these three patients had an increase in eosinophil count.

8.3 Risk Management

Based on the clinical observations, the risk associated with the increase in liver aminotransferases observed with bosentan treatment can be reduced/managed by the following measures:

– The target maintenance dose should be 125 mg b.i.d.

– Monthly evaluation of liver aminotransferases should be performed for the first 6 months of treatment and quarterly thereafter. This can be incorporated into the patient's routine blood testing for INR and electrolytes (most patients with pulmonary arterial hypertension are treated with anticoagulants and diuretics).

– Clear guidelines should be established for dose reduction and/or stopping treatment in cases of elevated liver aminotransferases (see proposed guidelines in Appendix 23).

– Physicians, nurses, pharmacists and patients should receive detailed information and education regarding the risk and treatment of an increase in liver aminotransferases.

9 EFFECTS OF BOSENTAN ON HEMOGLOBIN CONCENTRATION

The effects of bosentan treatment on RBC parameters have been investigated in both *in vitro* and *in vivo* experiments and by a careful examination of the clinical database. The evaluation has focused on the decrease in hemoglobin concentration, which is representative of similar changes observed in hematocrit and RBC count. This Section first summarizes the preclinical data (see also Section 3), followed by a description of the clinical findings and a discussion of the potential mechanism for the observed decreases in hemoglobin concentration.

9.1 Summary of Preclinical Findings

A mild decrease in RBC parameters (7% to 13%) in association with bosentan treatment was seen in preclinical and toxicology studies in dogs and rats. The decrease occurred during the first few weeks, and then concentrations stabilized during continued treatment. There was no evidence for hemolysis or immuno-allergic reaction (no increase in bilirubin or reticulocytes as both tended to decrease, no change in MCV or increase in the variability of erythrocyte size, and no increase in eosinophils, total IgG, or antibodies to bosentan), bone marrow toxicity, or bleeding tendency. However, there was evidence for a decrease in hemoglobin concentration and hematocrit secondary to an increase in plasma volume in rats treated with bosentan (see Section 3). Moreover, the decreases in RBC parameters were associated with a similar decrease (6% to 11%) in albumin concentration.

Based on these observations, hemodilution with fluid redistribution is the most likely explanation for this observation and is also compatible with the mechanism of action of the drug, i.e., vasodilation and reduction in vascular permeability.

9.2 Clinical Data on Bosentan-induced Decreases in Hemoglobin

In this report, decreases from baseline in hemoglobin concentration have been categorized in several ways. These categories include mild decreases of ≥ 1.0 g/dl, decreases to below the LLN, marked decreases in hemoglobin concentration (designated LL and defined as a decrease of ≥ 15% from baseline and to < 11.0 g/dl), and more pronounced decreases of ≥ 15% from baseline and to < 10.0 g/dl. In addition, mean changes from baseline to the end of treatment and to the maximal decrease during treatment have been summarized. Values for female patients have been transformed using the female and male reference ranges so that values can be combined (i.e., for evaluation of marked abnormalities). Sponsor-defined reference ranges are provided in Appendix 14.

9.2.1 Overall Incidence and Clinical Picture

In therapeutic studies, treatment with bosentan was associated with a modest decrease in hemoglobin concentration. The magnitude (placebo-corrected) of the decrease (overall and by dose) in the pool of placebo-controlled studies is described in Table 39, Appendix 24, and Appendix 25. Overall, mean hemoglobin concentrations decreased in bosentan-treated patients by about 0.8 g/dl (placebo-corrected change from baseline to both treatment end and to minimum value). The decrease was dose related.

Severity. A dose-related decrease in hemoglobin concentration of at least 1.0 g/dl was observed in more than 50% of bosentan-treated patients (56.8% vs 29.0% with placebo, Table 39 and Appendix 26). However, for a large proportion of these patients, hemoglobin remained within normal limits. The incidence of a marked decrease in hemoglobin concentration (LL, a decrease by 15% from baseline and to < 11.0 g/dl) was also dose related and greater among bosentan-treated patients than placebo-treated patients (overall incidence of 5.6% and 2.6%, respectively, see Appendix 28). In contrast, the incidence of a more pronounced marked decrease in hemoglobin of ≥ 15% from baseline and to < 10.0 g/dl was not dose related and was similar among bosentan- and placebo-treated patients (2.2% each, Table 39 and Appendix 29). Moreover, a clear reason for the decrease to < 10.0 g/dl could be identified in several cases in both treatment groups (e.g., epistaxis, gastrointestinal bleeding, surgery, and renal failure).

Table 39 **Placebo-corrected mean change from baseline and incidence of decreased hemoglobin concentration among bosentan-treated patients in placebo-controlled studies**

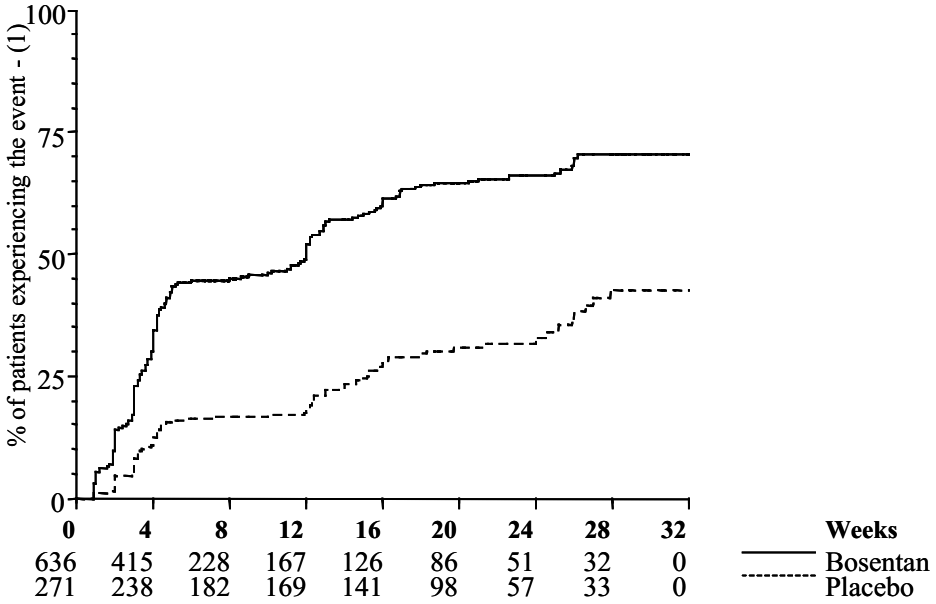
	Bosentan dose (mg/day)				
	100	250/500	1000/1500	2000	All doses
Change from baseline (g/dl)					
To treatment end [a]	−0.31	−0.80	−0.83	−0.93	−0.78
To minimum value during treatment [b]	−0.38	−0.95	−0.86	−0.89	−0.77
Percent of patients with a decrease in hemoglobin					
≥ 1.0 g/dl [c]	7.6%	31.2%	31.7%	42.9%	27.8%
To below the LLN [d]	2.0%	12.4%	17.2%	18.0%	7.5%
≥ 15% from baseline and to < 11.0 g/dl (LL) [e]	0	1.6%	6.6%	2.5%	3.1%
≥ 15% from baseline and to < 10.0 g/dl [f]	0	1.1%	0.6%	0	0

LLN = lower limit of normal.
For more detail see: [a] Appendix 24, [b] Appendix 25, [c] Appendix 26, [d] Appendix 27, [e] Appendix 28, [f] Appendix 29.

Time of onset. In the pool of placebo-controlled studies, the decrease in hemoglobin concentration occurred early in treatment for most patients (Figure 29). Most (92%) of the 36 cases of marked decrease in hemoglobin concentration (LL) among bosentan-treated patients were detected during the first 16 weeks of bosentan treatment (Figure 30). Similar observations were made in the ongoing, still blinded ENABLE study (Figure 31). In these trials, 1613 patients have been treated for at least 16 weeks, and 121 (7.5%) patients have had a marked decrease in hemoglobin concentration (LL) as of the clinical cut-off date (31 December 2000).

Figure 29 **Kaplan-Meier estimates of time to first decrease in hemoglobin concentration by at least 1.0 g/dl in placebo-controlled studies, safety population**

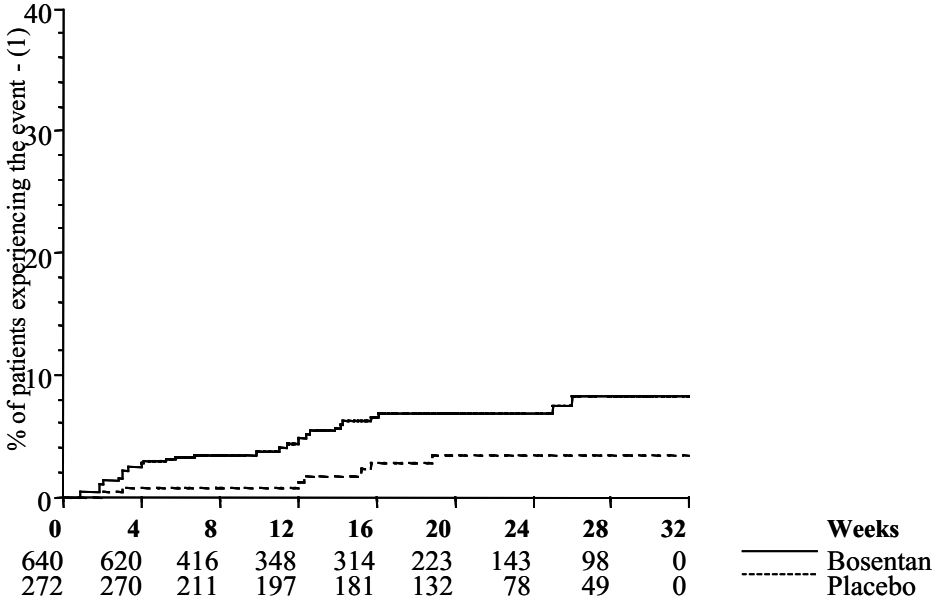
Time to Hemoglobin decrease >= 1 g 20JUN01
Studies: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018
NC-15020 NC-15462 NN-15031



Patients not experiencing the event are censored 28 days after the end of treatment.
(1) Kaplan Meier estimates

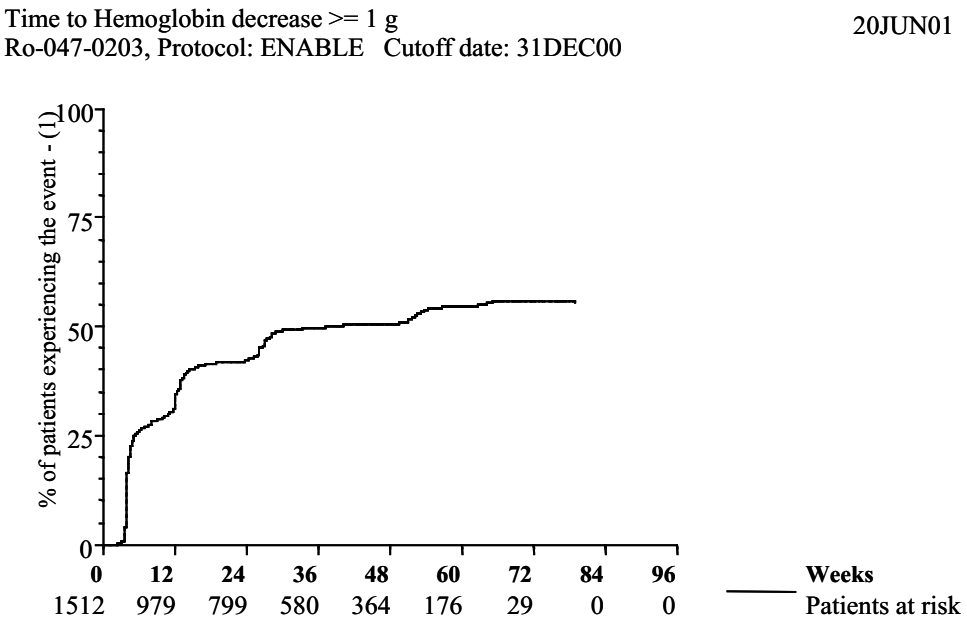
Figure 30 **Kaplan-Meier estimates of time to first appearance of a marked decrease in hemoglobin concentration (LL) in placebo-controlled studies, safety population**

Time to Hemoglobin LL 20JUN01
Studies: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018
NC-15020 NC-15462 NN-15031



Patients not experiencing the event are censored 28 days after the end of treatment.
(1) Kaplan Meier estimates

Figure 31 **Kaplan-Meier estimates of time to first decrease in hemoglobin concentration by at least 1.0 g/dl in the ENABLE study**



Only abnormalities after the baseline visits are considered.
(1) Kaplan Meier estimates

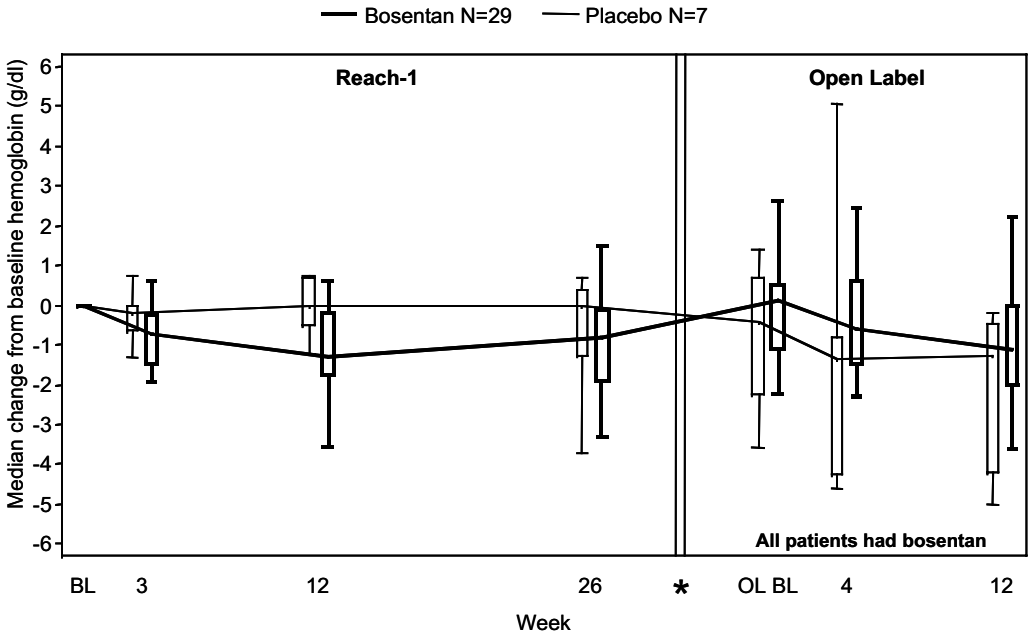
Effect over time. The time course of the decrease in hemoglobin concentration was evaluated in CHF patients and in those with pulmonary arterial hypertension.

In patients with severe CHF (Study NC15462), most of the decrease from baseline in mean hemoglobin concentration observed in bosentan-treated patients (500 mg b.i.d.) was already seen at the first laboratory evaluation at Week 3 (mean ± SD of −0.9 ± 0.9 g/dl). A slightly greater mean decrease from baseline was observed at Week 12 (–1.2 ± 1.3 g/dl). From Week 12 onward, hemoglobin concentrations stabilized or tended to increase. Thus, at Week 26 the mean decrease from baseline in hemoglobin concentration was similar to that observed at Week 3 (–0.9 ± 1.4 g/dl). These observations indicate that not only had most of the decrease occurred during the first few weeks of treatment, but the decrease in hemoglobin concentration was not progressive, and instead stabilized after 12 weeks of treatment.

This is further illustrated for the 29 bosentan- and 7 placebo-treated patients who entered the open-label extension trial (NC15464B) and had hemoglobin measurements at all time points. Several days to weeks after stopping treatment with bosentan 500 mg b.i.d. in the previous trial, these patients restarted treatment with bosentan 125 mg b.i.d.; and their hemoglobin concentration was measured after 4 and 12 weeks of treatment. The change in hemoglobin

concentrations over time for these patients during both trials is illustrated in Figure 32. Stopping treatment at the end of Study NC15462 resulted in an increase in hemoglobin concentration to pre-treatment levels. Both the restarting of bosentan treatment in ex-bosentan patients and the starting of bosentan treatment in ex-placebo patients in Study NC15464B resulted in a similar pattern of reduction in hemoglobin concentration (i.e., most of the decrease occurred during the first 4 weeks of treatment).

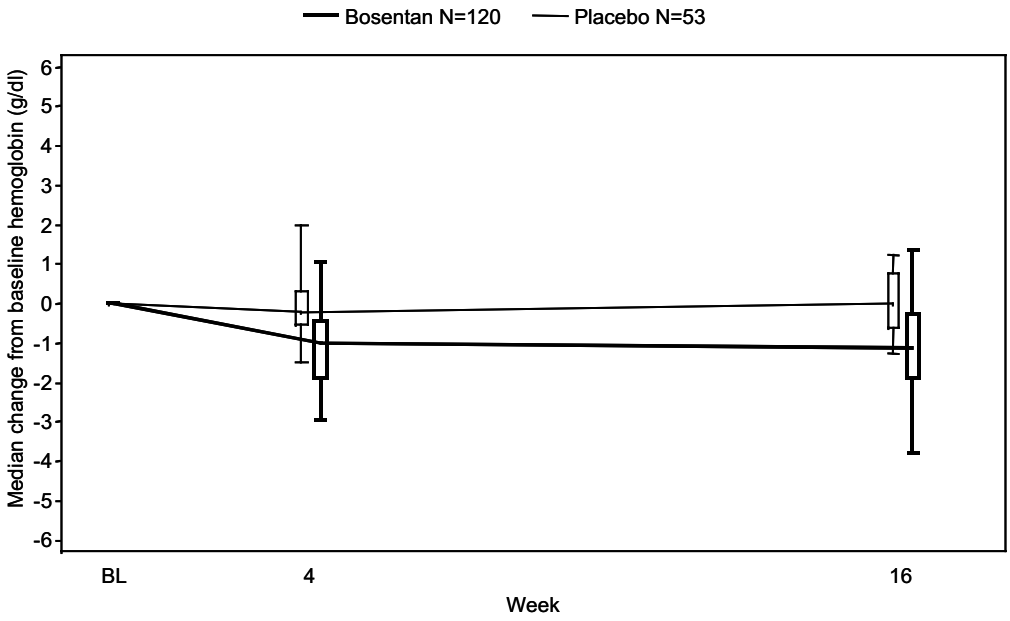
Figure 32 **Time course of changes in hemoglobin concentration, CHF patients in Study NC15462 (REACH-1) who continued in NC15464B and had measurements at all time points**



Median and 5th, 25th, 75th and 95th percentile
* All patients discontinued treatment at the end of REACH-1, and were started in the open-label study several weeks later.
BL = baseline, OL = open label.

The time course of the change in hemoglobin concentration in patients with pulmonary arterial hypertension in Study AC-052-352 is illustrated in Figure 33. Similar to the observations in CHF patients, the decreases in hemoglobin concentration occurred during the first 4 weeks of treatment and then remained stable.

Figure 33 **Time course of changes in hemoglobin concentration in Study AC-052-352 in pulmonary arterial hypertension, patients with measurements at all time points**



Median and 5th, 25th, 75th and 95th percentile

In the pool of placebo-controlled studies, hemoglobin concentration improved spontaneously (without blood transfusions and without treatment interruption) in 13 of the 36 bosentan-treated patients who had a marked decrease (LL). Hemoglobin concentration improved in 12 other patients after they had received blood transfusions or had treatment discontinued (not necessarily due to anemia). In two cases, the hemoglobin concentration remained low but stable, and one patient worsened. For eight patients no follow-up values were available.

Discontinuation of treatment because of anemia occurred in 5 (0.7%) bosentan-treated patients in the placebo-controlled studies. One CHF patient was also discontinued from the open-label study (NC15464B) due to anemia. None of the patients in the two pivotal trials in pulmonary arterial hypertension was discontinued for this reason. In the ongoing, still blinded ENABLE study, three patients have been discontinued due to anemia.

Confounding factors. There was no evidence of a relationship between the decrease in hemoglobin concentration and demographic factors (such as age, gender, race or weight). Patients with CHF and those with pulmonary arterial hypertension had a larger mean decrease in hemoglobin concentration and a higher incidence of marked decreases (LL) than did hypertensive patients. This could be related to the fact that patients with CHF or pulmonary arterial hypertension have fluid excess.

9.2.2 Blood Transfusions

In placebo-controlled studies, blood transfusions were given to 12 (1.8%) patients on bosentan and 3 (1.0%) on placebo. Four of the bosentan-treated patients who received blood transfusions had pulmonary arterial hypertension (Study AC-052-352), two with evidence for gastrointestinal bleeding, one with worsening of pre-existing anemia, and one with severe epistaxis. In Study NC15462 (REACH-1 in severe CHF), blood transfusions were given to 5 (2.0%) patients on bosentan and 2 (1.6%) on placebo. One of the bosentan-treated patients underwent bone marrow evaluation, which revealed normocellular marrow with adequate hematopoietic reserves. Four patients with SAH (3/47 treated with bosentan and 1/20 with placebo) received blood transfusions in relation to their surgery and hospital complications.

Five additional CHF patients received blood transfusions in the open-label study (NC15464B). The reasons for anemia and blood transfusion in these patients were metastatic melanoma, recurrent bleeding after urinary tract surgery, streptokinase treatment for unstable angina with positive occult blood, a large hematoma in a warfarin-treated patient, and pre-existing anemia in a patient who underwent polypectomy for gastric polyps during the previous double-blind trial (NC15462). None of these patients was discontinued due to anemia.

In the ENABLE study, 27 (1.7%) patients have received blood transfusions. In most cases it was related to episodes of bleeding, surgery, renal failure, or sepsis.

9.2.3 Possible Mechanisms

The safety database was evaluated in detail for evidence of any mechanism that could explain the observed decreases in hemoglobin. Possible reasons for the decrease include increased erythrocyte destruction (e.g., hemolysis), decreased erythrocyte production (e.g., bone marrow toxicity), increased loss (e.g., from bleeding) or none of the above (e.g., hemodilution).

Any sign of hemolysis?

One patient was diagnosed with Coombs positive hemolytic anemia in the ongoing open-label extension trial (AC-052-354) of Study AC-052-352 in pulmonary arterial hypertension. This 48-year-old female with primary pulmonary hypertension was known to have chronic anemia and tested positive for anti-nuclear antibodies and hepatitis B antibody. This combination of findings suggests that the patient could have an underlying autoimmune condition such as lupus erythematosus, and further tests are being done to investigate this (see Appendix 30 for full narrative).

In order to determine if there were any other cases of hemolysis, the following parameters were evaluated: changes in bilirubin concentration, MCV, and reticulocyte count. In addition, eosinophil count was evaluated as a signal for allergic reactions.

<u>*Total bilirubin*</u>. In the pool of placebo-controlled studies, mean bilirubin concentration decreased slightly from baseline to the end of treatment in bosentan-treated patients (−2.8 vs 0.3 µmol/l with placebo). The decrease in mean bilirubin concentration was greater in those bosentan-treated patients who had a marked decrease in hemoglobin concentration than in those whose hemoglobin concentration did not drop below the LLN (−3.6 vs −2.5 µmol/l, respectively).

Similar observations were made in the ENABLE study, i.e., mean bilirubin decreased slightly in the overall population (−0.5 µmol/l), and the decrease among patients with a marked decrease in hemoglobin was larger than among those whose concentration did not drop below the LLN (−0.8 vs −0.4 µmol/l, respectively).

None of the 36 bosentan-treated patients who had a marked decrease in hemoglobin concentration (LL) also had a marked increase in bilirubin concentration (HH, i.e., an increase by at least 50% from baseline to > 34.2 µmol/l). One patient had an increase in total bilirubin concentration to above the ULN (29.3 µmol/l, ULN = 17.1 µmol/l). However, this increase was associated with an increase in liver aminotransferases. In comparison, three placebo patients with marked decreases in hemoglobin (LL) also had an increase in bilirubin concentration to above the ULN, with one qualifying as a marked increase (HH).

These observations indicate that no clinically relevant increases in bilirubin concentration occurred among bosentan-treated patients. On the contrary, there was an overall tendency for bilirubin concentrations to decrease.

Reticulocyte count. Reticulocytes were not measured in the clinical trials included in the integrated database, but they were measured occasionally in the ongoing ENABLE study. Measurements both at baseline and during treatment are available for 285 patients, and in these patients, mean reticulocyte count decreased by 6.4×10^9/l. Mean reticulocyte count also decreased among the 26 of these patients who had a marked decrease in hemoglobin (LL), whereas it did not decrease among the 156 patients whose hemoglobin did not drop below the LLN (-10.8×10^9/l vs 1.7×10^9/l, respectively). An increase in reticulocyte count to ≥ 3% of the RBC count concomitant with a decrease in hemoglobin concentration was regarded as clinically relevant, and one patient met this criterion. This patient had an increase in reticulocytes from 1.7% (70×10^9/l) to 5.3% (175×10^9/l) of the RBC count and a decrease in hemoglobin concentration from 11.3 to 9.0 g/dl. There was no change in eosinophil count and no increase in bilirubin concentration. The patient was known to suffer from iron deficiency anemia, and he was treated with supplemental iron.

Mean corpuscular volume. MCV was measured in 1433 patients in the ENABLE study. Overall, mean values remained unchanged. Similarly, no changes were seen in patients who either did or did not have a marked decrease in hemoglobin concentration (LL).

Eosinophil count. Mean eosinophil count increased slightly (by 0.03×10^9/l from 0.17×10^9/l at baseline) among bosentan-treated patients (N = 585) compared with no change among placebo-treated patients (N = 252) in the pool of placebo-controlled trials. Among the 28 bosentan-treated patients with a marked decrease in hemoglobin concentration (LL), a larger mean increase in eosinophil count (by 0.09×10^9/l from 0.25×10^9/l at baseline) was observed than among those whose hemoglobin concentration did not drop to below the LLN (N = 409). However, only one of these patients had both a marked decrease in hemoglobin (LL) and a marked increase in eosinophils (HH, i.e., an increase by 100% from baseline to > 1.5×10^9/l). This patient had an eosinophil count of 0.67×10^9/l (8.6% of the WBC count) at baseline, which increased to 1.51×10^9/l (14.5%) and resolved to 0.33×10^9/l (4.5%) while treatment with bosentan continued.

This patient had scabies at baseline and was known to suffer from several drug allergies (e.g., sulfa, morphine). He also developed rash during the trial that prompted the discontinuation of captopril and furosemide. An increase in eosinophil counts to above the ULN (0.7×10^9/l) but below the level considered marked (HH) was observed in 6 of the 28 bosentan-treated patients and in one of the 7 placebo-treated patients who had a marked decrease in hemoglobin concentration (LL).

Conclusions. The absence of an increase in bilirubin in the placebo-controlled and ENABLE studies and the lack of a signal regarding reticulocytes and MCV in the ENABLE study, indicate that hemolysis is a very unlikely reason for the decrease in hemoglobin concentration among bosentan-treated patients. Although a mean increase in eosinophil count without other evidence for hemolysis among bosentan-treated patients cannot be explained, it is not thought to be a signal of an immuno-allergic reaction when it is an isolated laboratory finding.

Any sign of bone marrow toxicity?

The following parameters were evaluated: changes in WBC and platelet counts. Bone marrow evaluations were performed in two patients only.

White blood cell counts. Overall, WBC counts decreased slightly (by 0.7×10^9/l from 7.5×10^9/l at baseline) in bosentan-treated patients compared with no change in placebo-treated patients in the placebo-controlled studies. This decrease was slightly greater among bosentan-treated patients with markedly decreased hemoglobin (LL) than among those who did not have a decrease in hemoglobin concentration to below the LLN (-1.2 vs -0.5×10^9/l, respectively).

A marked decrease in WBC count was observed in similar proportions of patients in the bosentan and placebo groups (7/640 [1.1%] and 2/273 [0.7%], respectively). Marked decreases in hemoglobin concentration and WBC count were observed in one patient with pulmonary arterial hypertension and mixed connective tissue disease (Patient 105/10014, Study AC-052-352). Both of these parameters improved after treatment was stopped due to an increase in liver aminotransferases.

Platelet counts. In the pool of placebo-controlled studies, platelet counts increased in both bosentan- (by 2×10^9/l from 217×10^9/l at baseline) and placebo-treated patients (by 7×10^9/l from 219×10^9/l at baseline). Among bosentan-treated patients with a marked decrease in hemoglobin (LL), platelet counts increased (by 26×10^9/l from 244×10^9/l at baseline), and among those who did not have a decrease in hemoglobin concentration to below the LLN, it decreased (-6×10^9/l from 214×10^9/l at baseline).

A marked decrease in platelet count was observed in similar proportions of patients in the bosentan and placebo groups (3/600 [0.5%] and 2/262 [0.8%], respectively). Marked decreases in platelet count were not observed among patients with a marked decrease in hemoglobin (LL).

Four patients in the ENABLE study had pancytopenia. One patient was receiving chemotherapy for breast cancer, and one had myelodysplasia with chromosomal abnormalities. In another case, it was a transient observation in a patient who continued study treatment. The fourth patient had decreases in hemoglobin concentration (from 13.5 g/dl to 10.4 g/dl), WBC count (from

6.0×10^9/l to 2.9×10^9/l) and platelet count (from 107×10^9/l to 65×10^9/l). As a result, treatment was stopped, and all parameters improved.

Bone marrow evaluation. In Study NC15462, one patient (18202/9032) with a marked decrease in hemoglobin (from 12.5 g/dl to 9.6 g/dl) underwent bone marrow evaluation, which revealed normocellular marrow with adequate hematopoietic reserves. In the ENABLE study, another patient with a decrease in hemoglobin concentration (from 13.4 g/dl to 8.5 g/dl) underwent bone marrow evaluation, which revealed only iron deficiency anemia, confirming a pre-existing diagnosis. With iron supplement treatment, hemoglobin concentration increased to 11.1 g/dl.

Conclusion. These observations provide no evidence for bone marrow toxicity.

Any evidence for excess bleeding?

Bleeding tendency was assessed by evaluating bleeding-related AEs (preferred terms) such as gastrointestinal hemorrhage, melena, post-operative hemorrhage, epistaxis, hematuria, hemoptysis, menorrhagia, etc. Based on this approach, 29 (4.3%) of the 677 bosentan-treated patients and 14 (4.9%) of the 288 placebo-treated patients in placebo-controlled studies had evidence of bleeding.

Most patients with pulmonary arterial hypertension are treated with oral anticoagulants and/or antiplatelet medications. In the two placebo-controlled studies in these patients, bleeding events were reported by 21/165 (12.7%) and 11/80 (13.8%) bosentan- and placebo-treated patients, respectively.

Conclusion. These observations indicate that treatment with bosentan is not associated with an increased risk of bleeding.

9.3 Potential Reasons for the Decrease in Hemoglobin Concentration

Preclinical and clinical observations demonstrated that treatment with bosentan was associated with a reduction in hemoglobin concentration in the various studies in dogs and rats and in more than half of bosentan-treated patients. However, both preclinical evaluations and in-depth analysis of the clinical safety database did not reveal evidence for hemolysis, bone marrow toxicity or bleeding tendency. Two main mechanisms could explain these observations:

– Increased plasma volume and fluid shift due to vasodilation and decreased vascular permeability, resulting in hemodilution

– Reduction in erythropoietin levels

Endothelin-1 is a potent vasoconstrictor, and as such has experimentally been shown to be associated with a decrease in plasma volume and an increase in hematocrit without a change in RBC mass. These observations could also be related to the ability of ET-1 to increase capillary permeability. It is expected that these effects would be reversed by an endothelin antagonist such as bosentan, especially in cases of increased plasma ET concentrations (e.g., in severe CHF and pulmonary arterial hypertension). This effect is similar to that of other vasodilators such as ACE

inhibitors, alpha-adrenergic blockers and hydralazine, all of which have been associated with a reduction in hematocrit in relation to an expansion of the plasma volume [17].

As a dual ET receptor antagonist, bosentan prevents/reduces the ET-related increase in capillary permeability. The resultant increase in plasma volume with fluid shift can induce pseudo-anemia. This hypothesis is supported by the preclinical observations that bosentan treatment was associated with an increase in plasma volume and a decrease in hemoglobin concentration. However, in order to prove this hypothesis, plasma volume and RBC mass should be evaluated before and after the decrease in hemoglobin concentration. Stable RBC mass may be the only proof for the hypothesis, and this evaluation has not yet been done in humans.

Patients with severe CHF and low cardiac output have increased blood levels of erythropoietin. Treatment with bosentan may improve renal hemodynamics and relieve renal hypoxia, which could result in a decreased production of erythropoietin by the kidneys. This in turn could affect erythrocyte production by the bone marrow. This mechanism was demonstrated in severe CHF patients treated with ACE inhibitors [18]. However, while this hypothesis could explain the observation in patients with severe CHF or pulmonary arterial hypertension, it cannot explain the reduction in hemoglobin concentration in hypertensive patients. Moreover, the decreases in hemoglobin seen in the clinical trials were too rapid (within 3 to 4 weeks) to be explained solely by decreases in erythropoietin and RBC production.

Although a decrease in erythropoietin could play a role in the pathogenesis of the reduction in hemoglobin concentration, hemodilution is more compatible with the overall observations. It should be noted that hemodilution with decreased hematocrit has been shown to be beneficial in patients with CHF that could be due to improved rheologic properties of the circulating blood [19, 20].

9.4 Risk Management

Because in most cases the bosentan-related decrease in hemoglobin concentration is modest, stabilizes after the first few weeks of treatment, and is unlikely to reach levels that require blood transfusions, the risk to the patients is low. However, it is recommended that hemoglobin concentrations be checked after 1, 3, and 6 months of treatment. This will ensure that if a marked decrease in hemoglobin concentration occurs, it will be identified and the appropriate measures taken (e.g., further evaluation to determine the cause or specific treatment, if deemed necessary based on clinical judgment).

10 OVERALL SAFETY CONCLUSIONS AS IN SUMMARY

Based on a thorough evaluation of the integrated safety database, the following conclusions emerge regarding the use of bosentan in patients with pulmonary arterial hypertension:

– Treatment with bosentan was well tolerated over a wide range of doses and in all studied indications. Among the most common adverse events reported in the safety database, those associated with bosentan treatment were flushing (placebo-subtracted incidence of 4.9%), abnormal hepatic function (3.8%), leg edema (3.3%), headache (3.0%), and anemia (2.4%), all of which appeared to be dose related.

– There was no evidence for an effect of bosentan treatment on the incidences of death or serious adverse events. The proportion of patients who had treatment prematurely discontinued due to adverse experiences was greater in patients on bosentan than in those on placebo, mainly due to elevated liver enzymes.

– Treatment with bosentan at high dosages (1000–1500 mg/day) was associated with an increased incidence of worsening heart failure in patients with severe chronic heart failure during the first month of treatment. This observation is likely related to the high starting dose and the speed of up-titration in this study. Early worsening of chronic heart failure was not reported in patients with pulmonary arterial hypertension treated with the recommended dosing regimen.

– Treatment with bosentan is associated with increased risk of liver injury, which is related to the increased incidence of elevated liver aminotransferases. Although to a lesser extent, marked decrease in hemoglobin concentration is also an associated risk.

– No relevant treatment-emergent electrocardiographic changes or changes in quantitative electrocardiographic parameters were observed in bosentan-treated patients, even at very high oral doses.

– No clinically relevant change in heart rate was observed with bosentan at any dose evaluated. Small mean decreases in blood pressures were observed that were dose dependent. However, reports of hypotension, postural hypotension, syncope, and dizziness in the overall population were more frequent on placebo. In patients with pulmonary arterial hypertension, hypotension occurred more frequently on bosentan but was generally mild and did not result in treatment discontinuation.

– Rebound effects were not observed in patients with systemic hypertension and in those with severe chronic heart failure, even after withdrawal of very high dosages. However, due to lack of experience in patients with pulmonary arterial hypertension, treatment should be stopped gradually, if possible (i.e., dose reduction to half the target dose for 3 to 7 days before full discontinuation).

The two main safety concerns that arose from the thorough evaluation of the bosentan safety database were bosentan's association with increased incidences of elevated liver aminotransferases and decreased hemoglobin concentrations. The incidences of both these abnormal laboratory findings appeared to be dose related, but the timing, severity, and potential risk to the patient differed. However, the clinical characteristics of both the abnormalities allow for appropriate management and reduction in the risk to the patient.

11 RECOMMENDED DOSAGES

Based on the efficacy findings in patients with pulmonary arterial hypertension and the pharmacokinetic and safety findings in all clinical studies with bosentan, the dose recommendations for the treatment of pulmonary arterial hypertension are as follows:

– Initial starting dose is bosentan 62.5 mg b.i.d.

– After 4 weeks of initial treatment, the dose should be increased to 125 mg b.i.d., the maintenance dose.

No dose adjustment is required based on age, gender, race, or renal status.

An increased risk of elevated liver aminotransferases was observed in patients receiving concomitant therapy with glibenclamide (glyburide). Such patients should be followed closely, and liver function tests should be performed when initiating glibenclamide treatment. If increases in liver aminotransferases occur, alternatives to glibenclamide treatment should be considered.

Co-administration of bosentan and cyclosporine A should be used only when the potential benefits outweigh the risks, as the resulting increase in bosentan concentration may increase the risk of hypotension and/or elevated liver aminotransferases. Patients should be followed closely, and the maximum dose of bosentan should not exceed 62.5 mg b.i.d. Although there is no experience with tacrolimus, the potential exists for an interaction similar to that seen with cyclosporine A and, therefore, the same precautions should apply.

Bosentan should not be given to patients with moderate to severe liver impairment, to pregnant women, or to women who may become pregnant unless adequate contraceptive measures are taken. It is not known whether this drug is excreted in human milk, and therefore, bosentan should be discontinued when breast-feeding.

12 RISK / BENEFIT EVALUATION

Pulmonary arterial hypertension is a debilitating, progressive disease associated with poor quality of life and poor prognosis. Conventional therapy includes calcium channel blockers, oral anticoagulants and diuretics, but calcium channel blockers are effective in only a small percentage of patients. Epoprostenol is the only currently approved treatment known to be efficacious in patients with pulmonary arterial hypertension, but it must be delivered by continuous infusion into a central vein via an in-dwelling catheter. In addition to the inherent problems of the delivery system and possible life-threatening complications, e.g., sepsis and thrombosis, tolerance to the compound develops over time and abrupt withdrawal elicits considerable rebound effects. Therefore, an orally effective medication that improves exercise capacity and symptoms would add considerably to the quality of life of patients with pulmonary arterial hypertension.

Benefits of Bosentan in Pulmonary Arterial Hypertension

Results of the clinical studies indicate that bosentan is an effective oral therapy for the treatment of pulmonary arterial hypertension. Consistent improvements in all clinical and hemodynamic

parameters were obtained in both pivotal studies. Patients treated with bosentan were able to improve their exercise capacity as indicated by the statistically significant and clinically meaningful improvement in walk distance. This benefit was obtained in all subpopulations studied, regardless of demographics, disease characteristics, or values for baseline parameters. Moreover, the decrease in dyspnea during exercise, while patients were able to increase exercise capacity, indicates that treatment with bosentan is associated with symptom relief. Further support for symptom relief comes from the improvements in WHO functional class, a classification system based on the degree of dyspnea, fatigue, and limitations on physical activity. Being able to increase exercise capacity with less dyspnea even in the worst cases (WHO class IV) enables these patients to improve their daily lives.

Moreover, bosentan was found to significantly reduce the risk of clinical worsening, which suggests that the clinical course of the disease may be altered. This is further supported by the sustained benefits observed in 28 patients who have been treated with bosentan for at least 1 year in the open-label trial, as the improvements in WHO functional class were maintained, and none of the patients have died.

Moreover, although the number of patients treated for at least 1 year is small, none of the 28 patients with long-term treatment has died, which suggests improved survival compared to historical data.

Risks of Bosentan Therapy

Treatment with bosentan is generally well tolerated but is associated with risks related to an elevation in liver aminotransferases, a decrease in hemoglobin concentration, and potential teratogenic effects in the case of pregnancy.

The increases in liver aminotransferases associated with bosentan treatment are dose related and occur with an incidence of about 11%. They are typically asymptomatic, usually apparent within the first 4 months of treatment, and completely reversible upon treatment discontinuation. To date, there have been no cases of acute liver failure during treatment with bosentan or evidence of liver injury after treatment cessation. Although no patients strictly met the Zimmerman criteria for the assessment of the risk of acute liver failure, a conservative approach was taken, and the estimated risk of acute liver failure with bosentan was calculated to be 1/5000. This risk can be reduced by taking the appropriate precautionary measures, such as physician and patient education and frequent monitoring of liver enzymes.

More than 50% of patients treated with bosentan showed a decrease in hemoglobin concentration. In most patients, the decrease was modest, appeared during the first weeks of treatment, generally stabilized with continued treatment, and was unlikely to reach a level that required blood transfusions. Available evidence did not support hemolysis, bone marrow toxicity, or bleeding tendency as possible mechanisms. As with other vasodilators, the effect is most likely due to hemodilution. Although the risk to the patients is low, hemoglobin concentration should be monitored in order to ensure that if a marked decrease occurs, it will be identified and the appropriate measures taken (e.g., further evaluation to determine the cause or specific treatment, if deemed necessary based on clinical judgment).

Bosentan was shown to be teratogenic in preclinical experiments in animals, which is considered a characteristic of the drug class. No experience in humans exists in this regard, but it is considered likely that bosentan has teratogenic potential in humans and, therefore, strict measures should be taken to avoid pregnancy during treatment with bosentan.

Therapeutic Justification

As an oral therapy, bosentan represents a significant advance in the treatment of patients with pulmonary arterial hypertension. It offers convenient treatment with considerable clinical benefit to patients with life-threatening disease and few treatment options. Not only do the improvement in exercise capacity and relief of symptoms obtained with bosentan treatment contribute to an improved quality of life, but evidence suggests that treatment may affect the clinical course of the disease. The oral route of administration has clear advantages in terms of patient acceptability, ease of use, safety and tolerability over drugs that must be administered intravenously. The risks to patient well being associated with bosentan treatment has been characterized, quantified and can be reduced and effectively managed with appropriate precautionary measures and monitoring within the current treatment practices for these patients. Although important to follow, the recommended precautions do not present an undue burden for the patient that is likely to affect quality of life or lead to non-compliance.

Overall, oral treatment with bosentan at the recommended dosages was well tolerated and is associated with considerable benefits that outweigh the risks.

13 REFERENCES

1. Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, et al. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. N Engl J Med 1993; 328:1732-1739.

2. Saleh D, Furukawa K, Tsao MS, Maghazachi A, Corrin B, Yanagisawa M, et al. Elevated expression of endothelin-1 and endothelin-converting enzyme-1 in idiopathic pulmonary fibrosis: possible involvement of proinflammatory cytokines. Am J Respir Cell Mol Biol 1997; 16:187-193.

3. Galie N, Grigioni F, Bacchi-Reggiani K, Ussia GP, Parlangeli R, Catanzariti P, et al. Relation of endothelin-1 to survival in patients with primary pulmonary hypertension. Eur J Clin Invest 1996; 26 (suppl 1):273.

4. Ishikawa S, Miyauchi T, Ueno H, et al. Influence of pulmonary blood pressure and flow on endothelin-1 production in humans. J Cardiovasc Pharmacol 1995; 26:429-433.

5. Gaine SP, Rubin LJ. Primary pulmonary hypertension. Lancet 1998; 352:719-725.

6. Oakley C. Primary pulmonary hypertension: case series from the United Kingdom. Chest 1994; 105 (suppl):29S-32S.

7. Brenot F. Primary pulmonary hypertension: case series from France. Chest 1994; 105 (suppl):33S-36S.

8. Rich S, Kaufmann E, Levy PS. The effect of high doses of calcium-channel blockers on survival of primary pulmonary hypertension. N Engl J Med 1992; 327:76-81.

9. Wanstall JC, Jeffery TK. Recognition and management of pulmonary hypertension. Drugs 1998; 56:989-1007.

10. Barst RJ, Rubin LJ, Long WA, McGoon MD, Rich S, Badesch DB, et al. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. New Engl J Med 1996; 334:296-302.

11. Actelion Pharmaceuticals. Application for medicinal product designation. In.: Actelion Pharmaceuticals; 2000.

12. Fishman AP. Epoprostenol (prostacyclin) and pulmonary hypertension. Ann Intern Med 2000; 132:500-502.

13. Clozel M. Summary of preclinical pharmacology for bosentan (Ro 47-0203). Actelion Ltd. Research Report No. B-00.019, August 23, 2000.

14. D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. Ann Internal Med 1991; 115:343-349.

15. Zimmerman HJ. Hepatotoxicity - The adverse effects of drugs and other chemicals on the liver. Second ed. Philadelphia: Lippincott, 1999.

16. Bankowski Z, Begaud B, Benhamou JP, Benichou C, Bircher J, Danan G, et al. Criteria of drug-induced liver disorders. J Hepatol 1990; 11:272-276.

17. Wysocki M, Andersson OK, Persson B, Bagge U, Braide M. Hemorheologic effects of vasodilation in essential hypertension. Angiology 1996; 47:869-878.

18. Herrlin B, Nyquist O, Sylven D. Introduction of a reduction in hemoglobin concentration by enalapril in stable, moderate heart failure: a double-blind study. Br Heart J 1991; 66:199-205.

19. Feigenbaum MS, Welsch MA, Mitchell M, Vincent K, Braith RW, Pepine CJ. Contracted plasma and blood volume in chronic heart failure. J Am Coll Cardiol 2000; 35:51-55.

20. Besarab A, Bolton WK, Browne JK, Egrie JC, Nissenson AR, Okamoto DM, et al. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. N Engl J Med 1998; 339:584-590.

21. Guyatt GH, Sullivan MJ, Thompson JJ, Fallen EL, Pugsley SO, Taylor DW, et al. The 6-minute walk: a new measure of exercise capacity in patients with chronic heart failure. Can Med Assoc J 1985; 132:919-923.

14 APPENDICES

Appendix 1 Safety data from the exploratory study in pulmonary arterial hypertension (Study BD14884)

All seven patients in this study received an i.v. infusion of bosentan on Day 1 of the study (Part I). The dosage was 500 mg of bosentan (one patient received 200 mg), and patients had high plasma concentrations of bosentan. In association with the high dose i.v. infusion of bosentan, all patients reported AEs on Days 1 and 2 of the study. Adverse events that occurred in more than one patient include hypotension (5 patients), headache (3 patients), nausea/vomiting (2 patients), and dyspnea (2 patients), some of which were SAEs (see Section 7.7.2). During the oral phase of the study (bosentan 1000 mg b.i.d.), 2 patients on bosentan had a urinary tract infection (Days 12 and 16).

All seven patients also experienced at least one SAE. During or following the i.v. infusion, 4 patients experienced hypotension. Hypotension was one of several concomitant SAEs reported for the 2 patients randomized to placebo who died on Day 3 of the study (hypotension, thrombocytopenia, oliguria for Patient 03; hypotension, respiratory insufficiency, acute renal failure for Patient 07). Hypotension was the reason for reducing the i.v. dose in Patient 05, but the concomitant SAEs of hypotension, chest pain and dyspnea reported on Day 2 were resolved without sequelae, as was the hypotension reported on Day 1 for Patient 06. During treatment with oral bosentan 1000 mg b.i.d., SAEs of deterioration of the primary disease (Patient 02, Day 55), gastrointestinal disorder (Patient 04, Day 35), and circulatory failure/lung infection (Patient 05, Day 30) were reported. Patient 05 was prematurely withdrawn during Week 8 of treatment because of hospitalization due to the chest infection and because the study had terminated. Patient 01 (placebo treatment) was found to have breast carcinoma on Day 8.

The study was stopped when two of the three patients randomized to placebo in Part II died on Day 3 of the study. Both patients were severely ill and were non-responders to inhaled nitric oxide. For Patient 03, the cause of death was unclear as no autopsy was performed, and sepsis could not be ruled out. This patient had high pulmonary pressure and resistance at baseline and although she responded to i.v. bosentan treatment, the prolonged hypotension resulting from the high i.v. dose could have contributed to the clinical deterioration. For Patient 07, autopsy results revealed pulmonary edema and bilateral pleural effusions, and the cause of death was given as cardiac failure due to pulmonary hypertension. The withdrawal of calcium channel blockers 24 hours prior to study start and/or the withdrawal of the high dose of bosentan could have resulted in rebound pulmonary hypertension and contributed to the clinical deterioration. None of the 4 patients that were randomized to oral bosentan in this study died.

During the i.v. infusions, both systolic and diastolic blood pressures decreased in a dose-dependent manner, which was associated with a slight increase in heart rate. During the oral phase of the trial (2000 mg/day), blood pressure did not further decrease and heart rate remained slightly elevated in the four bosentan-treated patients.

All of these patients had ECG abnormalities at baseline, but no clinically relevant treatment-emergent ECG changes or changes in heart rate, PQ-, QRS-, or QT-intervals were observed.

Appendix 2 Borg dyspnea index scale

Perceived Breathlessness (Borg scale)

0	**NOTHING AT ALL**
0.5	**VERY VERY SLIGHT (just noticeable)**
1	**VERY SLIGHT**
2	**SLIGHT**
3	**MODERATE**
4	**SOMEWHAT SEVERE**
5	**SEVERE**
6	
7	**VERY SEVERE**
8	
9	**VERY VERY SEVERE (almost maximum)**
10	**MAXIMUM**

This procedure is described by Guyatt et al.[21].

Appendix 3 Borg dyspnea index: Change from baseline to end of Period 1 in Study AC-052-352, ITT population

Produced by sturlor on 04MAY01

Ro 47-0203, Protocol: AC-052-352
Table T10 Borg dyspnea index: change from baseline to week 16
Population: ITT

Borg dyspnea index

	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
Baseline				
n	74	70	144	69
Mean	3.3	3.8	3.6	3.8
Standard deviation	2.2	1.9	2.0	2.0
95% CL of mean	2.8 , 3.8	3.4 , 4.3	3.2 , 3.9	3.4 , 4.3
Median	3.0	4.0	3.0	4.0
95% CL of median	3.0 , 4.0	3.0 , 4.0	3.0 , 4.0	3.0 , 4.0
Min , Max	0.0 , 10.0	0.0 , 9.0	0.0 , 10.0	0.0 , 10.0
Week 16				
n	74	70	144	69
Mean	3.3	3.3	3.3	4.2
Standard deviation	2.7	2.3	2.5	2.4
95% CL of mean	2.6 , 3.9	2.7 , 3.8	2.9 , 3.7	3.6 , 4.8
Median	3.0	3.0	3.0	4.0
95% CL of median	2.0 , 3.0	3.0 , 3.0	3.0 , 3.0	3.0 , 4.0
Min , Max	0.0 , 10.0	0.0 , 9.0	0.0 , 10.0	0.0 , 10.0
Change from baseline				
n	74	70	144	69
Mean	-0.1	-0.6	-0.3	0.3
Standard deviation	2.1	1.9	2.0	2.0
95% CL of mean	-0.5 , 0.4	-1.0 , -0.1	-0.6 , 0.0	-0.2 , 0.8
Median	0.0	-0.3	0.0	0.0
95% CL of median	0.0 , 0.0	-1.0 , 0.0	0.0 , 0.0	0.0 , 0.0
Min , Max	-6.5 , 6.0	-6.0 , 4.0	-6.5 , 6.0	-3.0 , 7.0
TREATMENT EFFECT				
Mean	-0.4	-0.9	-0.6	
95% CL of mean	-1.1 , 0.3	-1.6 , -0.2	-1.2 , -0.1	
Median	-0.2	-0.7	-0.3	
95% CL of median	-0.8 , 0.4	-1.4 , -0.0	-1.0 , 0.1	

CL=confidence limits.
(Page 1/1)

Appendix 4 Time from randomization to clinical worsening in Study AC-052-352, ITT population

Produced by sturlor on 06JUL01

Ro 47-0203, Protocol: AC-052-352
Table T12a Time from randomization to clinical worsening
Population: ITT

K-M estimate of the event-free rate (%)	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
At 8 weeks (56 days)				
Patients at risk	71	70	141	63
Patients censored	-	-	-	-
Patients with event	3	-	3	6
K-M estimate (%)	95.9	100	97.9	91.3
95% confidence interval (%)	91.5 , 100	100.0 , 100	95.6 , 100	84.7 , 98.0
At 16 weeks (112 days)				
Patients at risk	55	48	103	48
Patients censored	18	23	41	18
Patients with event	4	3	7	10
K-M estimate (%)	94.6	95.3	95.0	85.2
95% confidence interval (%)	89.4 , 99.7	90.2 , 100	91.4 , 98.6	76.7 , 93.7
End of study				
Patients at risk	-	-	-	-
Patients censored	69	66	135	55
Patients with event	5	4	9	14
K-M estimate (%)	88.3	90.0	89.3	62.9
95% confidence interval (%)	75.4 , 100	78.8 , 100	80.6 , 97.9	41.2 , 84.7
Treatment difference				
Logrank p-value vs. placebo	0.0133	0.0122	0.0015	

Patients were censored at the date of trial treatment end plus 1 day.
K-M = Kaplan-Meier.
(Page 1/1)

Appendix 5 Time from randomization to clinical worsening in Study AC-052-352 (Period 2 patients censored at Week 16), ITT population

Produced by sturlor on 06JUL01

Ro 47-0203, Protocol: AC-052-352
Table T12b Time from randomization to clinical worsening in period 1
Population: ITT

K-M estimate of the event-free rate (%)	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
At 8 weeks (56 days)				
Patients at risk	71	70	141	63
Patients censored	-	-	-	-
Patients with event	3	-	3	6
K-M estimate (%)	95.9	100	97.9	91.3
95% confidence interval (%)	91.5 , 100	100.0 , 100	95.6 , 100	84.7 , 98.0
End of period 1				
Patients at risk	-	-	-	-
Patients censored	70	67	137	57
Patients with event	4	3	7	12
K-M estimate (%)	94.6	95.2	94.9	75.2
95% confidence interval (%)	89.4 , 99.7	89.9 , 100	91.3 , 98.6	58.6 , 91.7
Treatment difference				
Logrank p-value vs. placebo	0.0298	0.0197	0.0038	

Patients who entered period 2 were censored at the date of the week 16 visit, otherwise at the date of trial treatment end plus 1 day.
K-M = Kaplan-Meier.
(Page 1/1)

Appendix 6 Incidence of clinical worsening to Week 28 in Study AC-052-352, ITT population

Produced by sturlor on 04MAY01

Ro 47-0203, Protocol: AC-052-352
Table T13 Incidence of clinical worsening from randomization to week 28
Population: ITT

	Bosentan 125 mg N=74		Bosentan 250 mg N=70		All Bosentan N=144		Placebo N=69	
	No.	%	No.	%	No.	%	No.	%
Patients with clinical worsening*	5	6.8%	4	5.7%	9	6.3%	14	20.3%
Death	1	1.4%	-		1	0.7%	2	2.9%
Hospitalization for PHT	3	4.1%	3	4.3%	6	4.2%	9	13.0%
Lack of clinical/walk test improvement	-		-		-		1	1.4%
Worsening of patient condition	3	4.1%	2	2.9%	5	3.5%	5	7.2%
Need for prostacyclin	2	2.7%	2	2.9%	4	2.8%	3	4.3%

* Patients may fall into more than one category.
(Page 1/1)

Appendix 7 WHO functional class: Number of patients improved at the end of Period 1 in Study AC-052-352, ITT population

Produced by sturlor on 04MAY01

Ro 47-0203, Protocol: AC-052-352
Table T11b WHO functional class: patients who improved during period 1
Population: ITT

Change at week 16	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
n	74	70	144	69
Improved [n (%)]	32 (43.2%)	29 (41.4%)	61 (42.4%)	21 (30.4%)
95% confidence limits (%)	31.8 , 55.3	29.8 , 53.8	34.2 , 50.9	19.9 , 42.7
Treatment effect*				
Difference	12.8%	11.0%	11.9%	
95% confidence limits (%)	-4.0 , 28.6	-5.9 , 27.0	-2.9 , 25.2	

* Compared with placebo
(Page 1/1)

Appendix 8 Change from baseline in central hemodynamic parameters in Study AC-052-351, ITT population

Produced by sturlor on 18AUG00

Ro 47-0203, Protocol: AC-052-351
Table T30: Change from baseline up to to week 12 in hemodynamic measurements
Population: ITT

MEAN PULMONARY ARTERY PRESSURE (mmHg)

	Bosentan	Placebo
	N=21	N=11
Baseline		
n	20	10
Mean	53.7	55.7
SD	13.4	10.5
Stderr	3.0	3.3
Median	51.3	57.3
Min , Max	35.5 , 82.5	39 , 72
Week 12(*)		
n	20	10
Mean	52.1	60.8
SD	12.6	13.3
Stderr	2.8	4.2
Median	50.8	63.0
Min , Max	31 , 79	36.5 , 79
Absolute change		
n	20	10
Mean	-1.6	5.1
SD	5.1	8.8
Stderr	1.2	2.8
95% CL of mean	-4.0 , 0.8	-1.2 , 11.4
Median	-2.0	4.8
95% CL of median	-4.5 , 0.5	-4.0 , 14.0
Min , Max	-10.5 , 9.5	-4.5 , 24
Percent change		
n	20	10
Mean	-2.5	9.4
SD	10.6	15.9
Stderr	2.4	5.0
Median	-3.3	8.1
Min , Max	-22.3 , 23.2	-7.9 , 43.6

DIFFERENCE FROM PLACEBO

Absolute change	
Mean	-6.7
Stderr	2.5
95% CL of mean	-11.9 , -1.5
Median	-5.8
95% CL of median	-11.6 , -0.4
p-value t-test	0.0134
p-value Mann-Whitney U-test	0.0300

(*) Patients prematurely withdrawn in period I are assigned worst rank when no assessment at week 12 or at premature withdrawal is available.

Appendix 8 Change from baseline in central hemodynamic parameters in Study AC-052-351, ITT population (cont.)

Produced by sturlor on 18AUG00

Ro 47-0203, Protocol: AC-052-351
Table T30: Change from baseline up to to week 12 in hemodynamic measurements
Population: ITT

PULMONARY VASCULAR RESISTANCE (dyn*sec/cm^5)

	Bosentan	Placebo
	N=21	N=11
Baseline		
n	19	10
Mean	896	942
SD	425	430
Stderr	97	136
Median	790	1042
Min , Max	309 , 1651	274 , 1544
Week 12(*)		
n	19	10
Mean	673	1133
SD	282	444
Stderr	65	140
Median	562	1223
Min , Max	258 , 1313	279 , 1600
Absolute change		
n	19	10
Mean	-223	191
SD	245	235
Stderr	56	74
95% CL of mean	-341 , -106	23 , 359
Median	-150	229
95% CL of median	-315 , -61	-14 , 453
Min , Max	-875 , 129	-224 , 487
Percent change		
n	19	10
Mean	-19.9	27.1
SD	21.1	34.0
Stderr	4.8	10.7
Median	-20.9	20.7
Min , Max	-61.6 , 29.7	-20.1 , 90.3

DIFFERENCE FROM PLACEBO

Absolute change	
Mean	-415
Stderr	94
95% CL of mean	-608 , -221
Median	-377
95% CL of median	-576 , -209
p-value t-test	0.0002
p-value Mann-Whitney U-test	0.0001

(*) Patients prematurely withdrawn in period I are assigned worst rank when no assessment at week 12 or at premature withdrawal is available.

Appendix 8 Change from baseline in central hemodynamic parameters in Study AC-052-351, ITT population (cont.)

Produced by sturlor on 18AUG00

Ro 47-0203, Protocol: AC-052-351
Table T30: Change from baseline up to to week 12 in hemodynamic measurements
Population: ITT

CARDIAC INDEX (l/min/m^2)

	Bosentan	Placebo
	N=21	N=11
Baseline		
n	20	10
Mean	2.35	2.48
SD	0.73	1.03
Stderr	0.16	0.33
Median	2.30	2.16
Min , Max	1.27 , 3.94	1.42 , 4.48
Week 12(*)		
n	20	10
Mean	2.85	1.96
SD	0.55	0.85
Stderr	0.12	0.27
Median	2.95	1.51
Min , Max	1.42 , 4.11	1.15 , 3.52
Absolute change		
n	20	10
Mean	0.50	-0.52
SD	0.46	0.48
Stderr	0.10	0.15
95% CL of mean	0.28 , 0.71	-0.86 , -0.18
Median	0.46	-0.67
95% CL of median	0.20 , 0.65	-0.93 , 0.11
Min , Max	-0.60 , 1.18	-1.24 , 0.20
Percent change		
n	20	10
Mean	26.4	-19.9
SD	25.6	18.6
Stderr	5.7	5.9
Median	19.3	-27.4
Min , Max	-15.3 , 79.6	-37.8 , 8.3
DIFFERENCE FROM PLACEBO		
Absolute change		
Mean	1.02	
Stderr	0.18	
95% CL of mean	0.65 , 1.39	
Median	1.04	
95% CL of median	0.65 , 1.38	
p-value t-test	<.0001	
p-value Mann-Whitney U-test	<.0001	

(*) Patients prematurely withdrawn in period I are assigned worst rank when no assessment at week 12 or at premature withdrawal is available.

Appendix 8 Change from baseline in central hemodynamic parameters in Study AC-052-351, ITT population (cont.)

Produced by sturlor on 18AUG00

Ro 47-0203, Protocol: AC-052-351
Table T30: Change from baseline up to to week 12 in hemodynamic measurements
Population: ITT

MEAN RIGHT ATRIAL PRESSURE (mmHg)

	Bosentan	Placebo
	N=21	N=11
Baseline		
n	19	10
Mean	9.7	9.9
SD	5.6	4.1
Stderr	1.3	1.3
Median	8.5	11.8
Min , Max	2.5 , 24.5	3.5 , 14
Week 12(*)		
n	19	10
Mean	8.5	14.8
SD	3.9	7.9
Stderr	0.9	2.5
Median	7.5	13.8
Min , Max	2 , 15.5	6 , 24.5
Absolute change		
n	19	10
Mean	-1.3	4.9
SD	4.1	4.6
Stderr	0.9	1.5
95% CL of mean	-3.2 , 0.7	1.6 , 8.2
Median	-0.5	3.5
95% CL of median	-3.5 , 0.5	0.0 , 11.0
Min , Max	-9 , 9	-0.5 , 12

DIFFERENCE FROM PLACEBO

Absolute change	
Mean	-6.2
Stderr	1.7
95% CL of mean	-9.6 , -2.7
Median	-5.5
95% CL of median	-10.2 , -2.1
p-value t-test	0.0010
p-value Mann-Whitney U-test	0.0010

(*) Patients prematurely withdrawn in period I are assigned worst rank when no assessment at week 12 or at premature withdrawal is available.

Appendix 8 Change from baseline in central hemodynamic parameters in Study AC-052-351, ITT population (cont.)

Produced by sturlor on 18AUG00

Ro 47-0203, Protocol: AC-052-351
Table T30: Change from baseline up to to week 12 in hemodynamic measurements
Population: ITT

PULMONARY CAPILLARY WEDGE PRESSURE (mmHg)

	Bosentan	Placebo
	N=21	N=11
Baseline		
n	19	10
Mean	9.3	8.3
SD	2.4	3.3
Stderr	0.6	1.1
Median	9.0	8.5
Min , Max	5 , 13	4 , 12
Week 12(*)		
n	19	10
Mean	9.4	12.2
SD	3.8	5.2
Stderr	0.9	1.6
Median	9.0	11.0
Min , Max	5 , 21	7 , 21
Absolute change		
n	19	10
Mean	0.1	3.9
SD	3.6	5.6
Stderr	0.8	1.8
95% CL of mean	-1.6 , 1.8	-0.1 , 7.9
Median	0.0	2.0
95% CL of median	-2.0 , 1.0	-1.0 , 10.0
Min , Max	-4 , 12	-1 , 17

DIFFERENCE FROM PLACEBO

Absolute change	
Mean	-3.8
Stderr	1.7
95% CL of mean	-7.3 , -0.3
Median	-2.8
95% CL of median	-5.9 , -0.2
p-value t-test	0.0353
p-value Mann-Whitney U-test	0.0270

(*) Patients prematurely withdrawn in period I are assigned worst rank when no assessment at week 12 or at premature withdrawal is available.

Appendix 9 Study designs for studies in other indications

Study BC15064 (severe CHF)

DAY

-14 to -1	1	2	7	14	15	22

Part I, Open-label

Bosentan 500 mg b.i.d. oral (n = 7)

Part II, Double-blind

Randomization

- Bosentan 1000 mg b.i.d. oral (n = 24)
- Placebo b.i.d. oral (n = 12)

Screening	Hospitalization			Hospitalization		Follow-up
Screening assessments	Invasive hemo-dynamics, safety, blood sampling	Safety assessments	Safety, blood sampling	Invasive hemo-dynamics, safety, blood sampling	Safety assessments	Safety assessments

Study NC15018 (severe CHF)

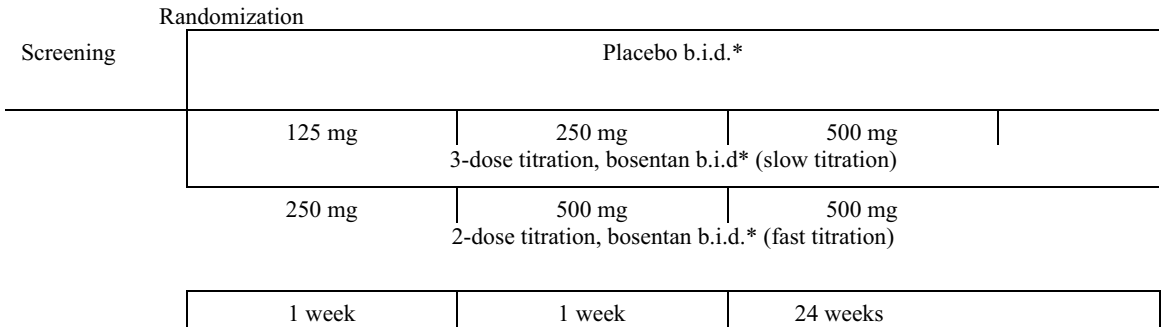
Day	-14 to -1	1 to 84	91
Procedure	Screening	Treatment	Follow-up

Randomization

- Bosentan group 1000 mg b.i.d. (n = 25)
- Placebo group (n = 9)

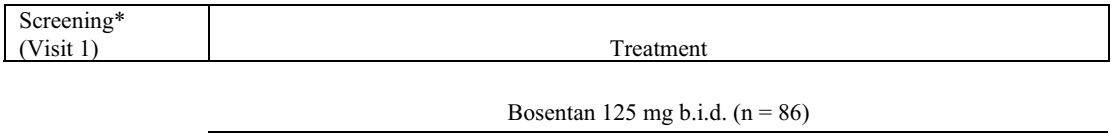
Appendix 9 Study designs for studies in other indications (cont.)

Study NC15462 (severe CHF)



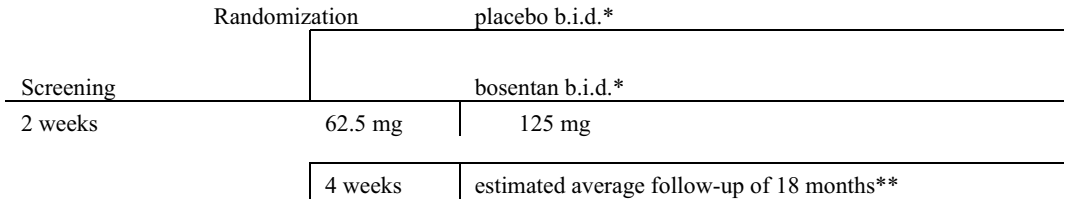
* The dose of trial medication was increased in all patients unless the patient experienced drug-related AEs. Dose reductions were allowed.

Study NC15464B (open-label extension of NC15462)



* Patients who are candidates for long-term, open-label therapy had screening visit within 8 weeks after completion of double-blind Study NC15462.

ENABLE (AC-052-301 and AC-052-302, severe CHF)



* The dose of study medication is increased in all patients unless the patient experienced drug-related AEs.

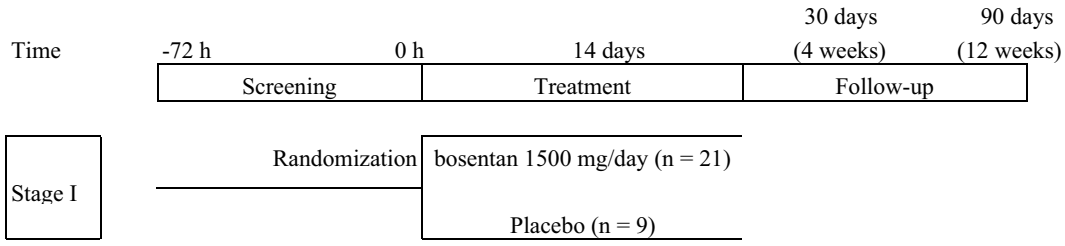
** The studies will be completed when all patients have been treated for ≥ 9 months (n = 750/ ENABLE trial) and a total of 600 deaths or hospitalizations for CHF have been recorded in both studies. The studies can be terminated early upon recommendation of the Data and Safety Monitoring Board.

Appendix 9 Study designs for studies in other indications (cont.)

Study NC15020 (systemic hypertension)

PERIOD I Placebo Run-in (4 – 6 weeks)		PERIOD II Active Treatment (4 weeks)	Follow-up (1 week)
placebo b.i.d.	Randomization	bosentan 100 mg q.d. (n = 50) bosentan 500 mg q.d. (n = 49) bosentan 1000 mg q.d. (n = 45) bosentan 1000 mg b.i.d. (n = 50) enalapril 20 mg q.d. (n = 50) placebo b.i.d. (n = 49)	

Study NN15031 (subarachnoid hemorrhage)



Appendix 10 Specific exclusion criteria

Factor	PAH		CHF				HTN	SAH
	AC-052-351 AC-052-352	**BD14884**	**NC15018**	**BC15064/II**	**NC15462**	**AC-052-301 AC-052-302**	**NC15020**	**NN15031**
Myocardial infarction, unstable angina pectoris	—	—	Within 12 wks	Within 12 wks	Within 6 wks	Within 4 wks	Within 24 wks	—
Cerebrovascular accident	—	—	Within 12 wks	Within 12 wks	Within 12 wks	Within 4 wks	Within 24 wks	Inclusion criteria
Systolic blood pressure (mmHg)	< 85	< 90	< 90	< 95	< 85	< 85	—	—
Heart rate (bpm)	—	—	—	—	< 50 or >130	< 50 or >130	—	—
Serum aminotransferases (× ULN)	> 3	—	> 1.5	> 1.5	> 3	> 3	> 1.5	—
Hemoglobin (outside the std ref range)	> 30%	—	—	—	> 30%	> 30%	—	—
Serum creatinine (mg/dl)	—	—	> 2.8	> 200 µmol/l	> 3.0	> 3.0	> 2.26	—

bpm = beats per minute; CHF = chronic heart failure; HTN = systemic hypertension; PAH = pulmonary arterial hypertension; SAH = subarachnoid hemorrhage; ULN = upper limit of normal.

Appendix 11 Disallowed concomitant medication

	PAH		CHF				HTN	SAH
Drug	**AC-052-351 AC-052-352**	**BD14884**	**NC15018**	**BC15064/II**	**NC15462**	**AC-052-301 AC-052-302**	**NC15020**	**NN15031**
CsA	X	—	—	—	X	X	—	—
Glibenclamide (glyburide)	X	—	—	—	—	X	—	—
Epoprostenol	X	—	—	—	—	—	—	—
Ca channel blockers	—	X*	—	Within 5 days	X	—	X	—
Anti-arrhythmics	—	—	X	Class I or III (excl. amiodarone)	—	—	X	—
Oral anticoagulants	—	X*	X	—	—	—	—	—
Beta blockers	—	—	X	Within 5 days	—	—	X	—
Others	only in Study AC-052-352: troglitazone, encainide, flecainide, disopyramide, propafenone, moricizine, pinacidil, minoxidil, oral positive inotropes (except digoxin)	CV meds other than diuretics*		long-acting nitrates tricyclic antidepressants phenothiazine H_2-blockers	encainide, flecainide, disopyramide, propafenone, moricizine, pinacidil, minoxidil, oral positive inotropes (except digoxin) (within 4 wks)	encainide, flecainide, disopyramide, propafenone, moricizine, pinacidil, minoxidil, oral positive inotropes (except digoxin) (within 4 wks)	vasodilators, ACE inhibitors, diuretics, guanethidine, reserpine, anti-sympathomimetics, oral contraceptives, psychotropics, anti-arthritic doses of NSAIDs	no restrictions on concomitant medications

* Medications were withdrawn prior to and during right heart catheterization but allowed otherwise during the trial.
Ca = calcium; CHF = chronic heart failure; CsA = cyclosporine A; CV = cardiovascular; HTN = hypertension; meds= medications; NSAIDs = nonsteroidal anti-inflammatory drugs; PAH = pulmonary arterial hypertension; SAH = subarachnoid hemorrhage.

Appendix 12 Summary of adverse events in placebo-controlled studies , safety population

Produced by sturlor on 17MAY01

Ro 47-0203, Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031
Table T31f: Summary of adverse events (including unrelated) by frequency
Population: Safety

Body system / Adverse event	Placebo N=288 No.	%	Bosentan N=677 No.	%
ALL BODY SYSTEMS				
Total pts with at least one AE	220	76.4%	529	78.1%
Total number of AEs	840		1591	
CARDIAC FAILURE NOS	64	22.2%	120	17.7%
HEADACHE NOS	37	12.8%	107	15.8%
DIZZINESS (EXC VERTIGO)	39	13.5%	80	11.8%
HYPOTENSION NOS	22	7.6%	46	6.8%
FLUSHING	5	1.7%	45	6.6%
HEPATIC FUNCTION ABNORMAL NOS	6	2.1%	40	5.9%
UPPER RESPIRATORY TRACT INFECTION NOS	18	6.3%	32	4.7%
OEDEMA LOWER LIMB	4	1.4%	32	4.7%
NAUSEA	30	10.4%	31	4.6%
DIARRHOEA NOS	18	6.3%	30	4.4%
CHEST PAIN NEC	20	6.9%	27	4.0%
DYSPNOEA NOS	14	4.9%	26	3.8%
COUGH	13	4.5%	26	3.8%
NASOPHARYNGITIS	10	3.5%	23	3.4%
ANAEMIA NOS	3	1.0%	23	3.4%
INFLUENZA	14	4.9%	20	3.0%
SYNCOPE	12	4.2%	20	3.0%
VISION BLURRED	7	2.4%	20	3.0%
BRONCHITIS NOS	10	3.5%	19	2.8%
URINARY TRACT INFECTION NOS	12	4.2%	18	2.7%
PALPITATIONS	5	1.7%	18	2.7%
BACK PAIN	8	2.8%	17	2.5%
VOMITING NOS	12	4.2%	16	2.4%
OEDEMA NOS	3	1.0%	16	2.4%
CONSTIPATION	7	2.4%	15	2.2%
ANGINA PECTORIS	3	1.0%	15	2.2%
FATIGUE	12	4.2%	14	2.1%
ARTHRALGIA	10	3.5%	14	2.1%
POSTURAL HYPOTENSION	14	4.9%	13	1.9%
ABDOMINAL PAIN NOS	11	3.8%	13	1.9%
PYREXIA	5	1.7%	13	1.9%
GOUT	7	2.4%	12	1.8%
PAIN IN LIMB	7	2.4%	12	1.8%
EPISTAXIS	5	1.7%	12	1.8%
LOWER RESPIRATORY TRACT INFECTION NOS	5	1.7%	12	1.8%
SINUSITIS NOS	5	1.7%	12	1.8%
PRURITUS NOS	-		12	1.8%
DYSPEPSIA	3	1.0%	11	1.6%
PNEUMONIA NOS	2	0.7%	11	1.6%
PULMONARY HYPERTENSION NOS AGGRAVATED	16	5.6%	10	1.5%
DERMATITIS NOS	5	1.7%	10	1.5%
ATRIAL FIBRILLATION	4	1.4%	10	1.5%
FACE OEDEMA	2	0.7%	10	1.5%
MUSCLE CRAMPS	6	2.1%	9	1.3%
HYPOKALAEMIA	2	0.7%	9	1.3%
DRY MOUTH	1	0.3%	9	1.3%
RESPIRATORY TRACT INFECTION NOS	4	1.4%	8	1.2%
VISUAL DISTURBANCE NOS	4	1.4%	8	1.2%
NASAL CONGESTION	2	0.7%	8	1.2%
RENAL FAILURE NOS	4	1.4%	7	1.0%
INSOMNIA NEC	6	2.1%	6	0.9%
GASTROENTERITIS NOS	3	1.0%	6	0.9%
RHINITIS NOS	2	0.7%	6	0.9%
INFLUENZA LIKE ILLNESS	1	0.3%	6	0.9%
APPETITE DECREASED NOS	3	1.0%	5	0.7%

Note: only treatment-emergent AEs are included.
(Pag 1/8)

Appendix 12 Summary of adverse events in placebo-controlled studies, safety population (cont.)

Produced by sturlor on 17MAY01
Ro 47-0203, Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031
Table T31f: Summary of adverse events (including unrelated) by frequency
Population: Safety

Body system / Adverse event	Placebo N=288 No.	%	Bosentan N=677 No.	%
CONTUSION	2	0.7%	5	0.7%
LETHARGY	2	0.7%	5	0.7%
RECTAL HAEMORRHAGE	-		5	0.7%
DIABETES MELLITUS NOS	3	1.0%	4	0.6%
HYPOGLYCAEMIA NOS	3	1.0%	4	0.6%
VERTIGO NEC	3	1.0%	4	0.6%
ABDOMINAL DISTENSION	2	0.7%	4	0.6%
DEHYDRATION	2	0.7%	4	0.6%
HAEMOPTYSIS	2	0.7%	4	0.6%
HYPERGLYCAEMIA NOS	2	0.7%	4	0.6%
ABDOMINAL PAIN UPPER	1	0.3%	4	0.6%
HERPES ZOSTER	1	0.3%	4	0.6%
HYPERTENSION NOS	1	0.3%	4	0.6%
ARRHYTHMIA NOS	-		4	0.6%
CONJUNCTIVITIS NOS	-		4	0.6%
ELECTIVE HEART TRANSPLANT	-		4	0.6%
GASTRO-OESOPHAGEAL REFLUX DISEASE	-		4	0.6%
HYPONATRAEMIA	-		4	0.6%
MYOCARDIAL INFARCTION	-		4	0.6%
TINNITUS	-		4	0.6%
HAEMATURIA	3	1.0%	3	0.4%
RENAL IMPAIRMENT NOS	3	1.0%	3	0.4%
TACHYCARDIA NOS	3	1.0%	3	0.4%
ANXIETY NEC	2	0.7%	3	0.4%
ASTHMA NOS	2	0.7%	3	0.4%
CYSTITIS NOS	2	0.7%	3	0.4%
EYE DISORDER NOS	2	0.7%	3	0.4%
PULMONARY OEDEMA NOS	2	0.7%	3	0.4%
ANGINA UNSTABLE	1	0.3%	3	0.4%
BRADYCARDIA NOS	1	0.3%	3	0.4%
BRONCHITIS ACUTE NOS	1	0.3%	3	0.4%
ECZEMA NOS	1	0.3%	3	0.4%
ERYTHEMA NEC	1	0.3%	3	0.4%
LOCALISED INFECTION	1	0.3%	3	0.4%
OEDEMA PERIPHERAL	1	0.3%	3	0.4%
VIRAL INFECTION NOS	1	0.3%	3	0.4%
ASCITES	-		3	0.4%
FEELING COLD	-		3	0.4%
HYDROCEPHALUS NOS	-		3	0.4%
MOUTH ULCERATION	-		3	0.4%
PARAESTHESIA NEC	-		3	0.4%
SWEATING INCREASED	-		3	0.4%
VENTRICULAR EXTRASYSTOLES	-		3	0.4%
GASTRITIS NOS	5	1.7%	2	0.3%
FALL	4	1.4%	2	0.3%
NECK PAIN	4	1.4%	2	0.3%
TOOTHACHE	4	1.4%	2	0.3%
HYPERSENSITIVITY NOS	3	1.0%	2	0.3%
MYALGIA	3	1.0%	2	0.3%
SINUS CONGESTION	3	1.0%	2	0.3%
SORE THROAT NOS	3	1.0%	2	0.3%
ABDOMINAL PAIN LOWER	2	0.7%	2	0.3%
ASTHENIA	2	0.7%	2	0.3%
HYPOAESTHESIA	2	0.7%	2	0.3%
CATARACT EXTRACTION	1	0.3%	2	0.3%
CENTRAL NERVOUS SYSTEM DEPRESSION NOS	1	0.3%	2	0.3%
EARACHE	1	0.3%	2	0.3%
INFECTION NOS	1	0.3%	2	0.3%
IRRITABILITY	1	0.3%	2	0.3%

Note: only treatment-emergent AEs are included.
(Pag 2/8)

Appendix 12 Summary of adverse events in placebo-controlled studies, safety population (cont.)

Produced by sturlor on 17MAY01
Ro 47-0203, Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031
Table T31f: Summary of adverse events (including unrelated) by frequency
Population: Safety

Body system / Adverse event	Placebo N=288 No.	%	Bosentan N=677 No.	%
MUSCULOSKELETAL PAIN	1	0.3%	2	0.3%
PAIN IN JAW	1	0.3%	2	0.3%
PAIN NOS	1	0.3%	2	0.3%
RESPIRATORY FAILURE (EXC NEONATAL)	1	0.3%	2	0.3%
SKIN DISORDER NOS	1	0.3%	2	0.3%
TREMOR NEC	1	0.3%	2	0.3%
WEIGHT DECREASED	1	0.3%	2	0.3%
WHEEZING	1	0.3%	2	0.3%
ATRIOVENTRICULAR BLOCK COMPLETE	-		2	0.3%
BREAST ENGORGEMENT	-		2	0.3%
BURSITIS	-		2	0.3%
CELLULITIS	-		2	0.3%
CORONARY ARTERY DISEASE NOS	-		2	0.3%
DIZZINESS POSTURAL	-		2	0.3%
DYSURIA	-		2	0.3%
EYE IRRITATION	-		2	0.3%
FLUID RETENTION	-		2	0.3%
HAEMATOCRIT DECREASED	-		2	0.3%
HAEMOGLOBIN DECREASED	-		2	0.3%
HAEMORRHOIDS	-		2	0.3%
INJURY NOS	-		2	0.3%
INTESTINAL OBSTRUCTION NOS	-		2	0.3%
JOINT SWELLING	-		2	0.3%
LACERATION	-		2	0.3%
LOOSE STOOLS	-		2	0.3%
MUSCLE TWITCHING	-		2	0.3%
NIGHTMARES	-		2	0.3%
PNEUMOTHORAX NOS	-		2	0.3%
SKIN ULCER NOS	-		2	0.3%
SOMNOLENCE	-		2	0.3%
URTICARIA NOS	-		2	0.3%
VERTIGO POSITIONAL	-		2	0.3%
DEPRESSION NEC	5	1.7%	1	0.1%
CEREBROVASCULAR ACCIDENT NOS	4	1.4%	1	0.1%
WEAKNESS	4	1.4%	1	0.1%
FLATULENCE	3	1.0%	1	0.1%
ORTHOPNOEA	3	1.0%	1	0.1%
VENTRICULAR TACHYCARDIA	3	1.0%	1	0.1%
ALOPECIA	2	0.7%	1	0.1%
ANOREXIA	2	0.7%	1	0.1%
BONE PAIN	2	0.7%	1	0.1%
BRONCHOSPASM NOS	2	0.7%	1	0.1%
COAGULATION TIME NOS PROLONGED	2	0.7%	1	0.1%
GASTROINTESTINAL HAEMORRHAGE NOS	2	0.7%	1	0.1%
GASTROINTESTINAL UPSET	2	0.7%	1	0.1%
LOIN PAIN	2	0.7%	1	0.1%
OTITIS MEDIA NOS	2	0.7%	1	0.1%
TOOTH ABSCESS	2	0.7%	1	0.1%
ACCIDENTAL OVERDOSE (THERAPEUTIC AGENT)	1	0.3%	1	0.1%
BLOOD IN STOOL	1	0.3%	1	0.1%
CANDIDA NOS	1	0.3%	1	0.1%
COAGULATION TIME NOS ABNORMAL	1	0.3%	1	0.1%
COAGULATION TIME NOS SHORTENED	1	0.3%	1	0.1%
CONVULSIONS NOS	1	0.3%	1	0.1%
DRY SKIN	1	0.3%	1	0.1%
ECCHYMOSIS	1	0.3%	1	0.1%
FEELING ABNORMAL	1	0.3%	1	0.1%
FOOT FRACTURE	1	0.3%	1	0.1%

Note: only treatment-emergent AEs are included.
(Pag 3/8)

Appendix 12 Summary of adverse events in placebo-controlled studies, safety population (cont.)

Produced by sturlor on 17MAY01
Ro 47-0203, Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031
Table T31f: Summary of adverse events (including unrelated) by frequency
Population: Safety

Body system / Adverse event	Placebo N=288 No.	%	Bosentan N=677 No.	%
HEARING IMPAIRED	1	0.3%	1	0.1%
HEPATIC CONGESTION	1	0.3%	1	0.1%
HEPATIC DISORDER NOS	1	0.3%	1	0.1%
HYPOVOLAEMIA	1	0.3%	1	0.1%
LIMB INJURY NOS	1	0.3%	1	0.1%
LUNG DISORDER NOS	1	0.3%	1	0.1%
MENORRHAGIA	1	0.3%	1	0.1%
NERVOUSNESS	1	0.3%	1	0.1%
OLIGURIA	1	0.3%	1	0.1%
ORAL CANDIDIASIS	1	0.3%	1	0.1%
POLYURIA	1	0.3%	1	0.1%
PRODUCTIVE COUGH	1	0.3%	1	0.1%
RASH PRURITIC	1	0.3%	1	0.1%
RHINORRHOEA	1	0.3%	1	0.1%
SKIN FUNGAL INFECTION NOS	1	0.3%	1	0.1%
SKIN LESION NOS	1	0.3%	1	0.1%
THROMBOCYTOPENIA	1	0.3%	1	0.1%
VASOVAGAL ATTACK	1	0.3%	1	0.1%
ACCIDENT AT HOME	-		1	0.1%
ACCIDENT NOS	-		1	0.1%
ADENOCARCINOMA NOS	-		1	0.1%
AMENORRHOEA NOS	-		1	0.1%
ANAL FISTULA	-		1	0.1%
ANAPHYLACTIC SHOCK	-		1	0.1%
ANGIOGRAM NOS	-		1	0.1%
AORTIC ANEURYSM	-		1	0.1%
ARTHRITIS NOS AGGRAVATED	-		1	0.1%
ASPIRATION	-		1	0.1%
BIPOLAR DISORDER NEC	-		1	0.1%
BLOOD ALKALINE PHOSPHATASE NOS INCREASED	-		1	0.1%
BLOOD LACTATE DEHYDROGENASE INCREASED	-		1	0.1%
BLOOD THYROID STIMULATING HORMONE ABNORMAL NOS	-		1	0.1%
BLOOD UREA INCREASED	-		1	0.1%
BREAST INFECTION NOS	-		1	0.1%
BRONCHOPNEUMONIA NOS	-		1	0.1%
CALCULUS RENAL NOS	-		1	0.1%
CARDIAC ARREST	-		1	0.1%
CATARACT UNILATERAL	-		1	0.1%
CATARRH	-		1	0.1%
CHEST PAIN (NON-CARDIAC)	-		1	0.1%
CONDITION AGGRAVATED	-		1	0.1%
CONJUNCTIVAL HAEMORRHAGE	-		1	0.1%
CONJUNCTIVITIS ALLERGIC	-		1	0.1%
DERMATITIS FUNGAL NOS	-		1	0.1%
DIABETES INSIPIDUS	-		1	0.1%
DIFFICULTY IN MICTURITION	-		1	0.1%
DISTURBANCE IN ATTENTION NEC	-		1	0.1%
DIVERTICULITIS	-		1	0.1%
DRY THROAT	-		1	0.1%
DUODENAL ULCER	-		1	0.1%
EAR CANAL STENOSIS NOS	-		1	0.1%
EAR INFECTION NOS	-		1	0.1%
ECTOPIC PREGNANCY	-		1	0.1%
EOSINOPHIL COUNT INCREASED	-		1	0.1%
ERYTHEMA MULTIFORME	-		1	0.1%
EYE ABNORMALITY NOS	-		1	0.1%
EYE INFLAMMATION NOS	-		1	0.1%

Note: only treatment-emergent AEs are included.
(Pag 4/8)

Appendix 12 Summary of adverse events in placebo-controlled studies, safety population (cont.)

Produced by sturlor on 17MAY01

Ro 47-0203, Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031
Table T31f: Summary of adverse events (including unrelated) by frequency
Population: Safety

Body system / Adverse event	Placebo N=288 No.	%	Bosentan N=677 No.	%
EYE PAIN	-		1	0.1%
FEELING HOT	-		1	0.1%
FEMORAL ARTERY ANEURYSM	-		1	0.1%
FEMORAL NECK FRACTURE	-		1	0.1%
FUNGAL INFECTION NOS	-		1	0.1%
GASTROENTERITIS VIRAL NOS	-		1	0.1%
GASTROINTESTINAL FUNGAL INFECTION	-		1	0.1%
GENITAL INFECTION FUNGAL NOS	-		1	0.1%
GINGIVAL BLEEDING	-		1	0.1%
GLUCOSE TOLERANCE IMPAIRED	-		1	0.1%
HEMIPARESIS	-		1	0.1%
HYPOREFLEXIA	-		1	0.1%
IMPOTENCE	-		1	0.1%
INFECTED SKIN ULCER	-		1	0.1%
INGUINAL HERNIA NOS	-		1	0.1%
INTERMENSTRUAL BLEEDING	-		1	0.1%
IRRITABLE BOWEL SYNDROME	-		1	0.1%
ISCHAEMIC FOOT	-		1	0.1%
JAUNDICE NOS	-		1	0.1%
JAW CYST	-		1	0.1%
JOINT STIFFNESS	-		1	0.1%
LABORATORY TEST ABNORMAL NOS	-		1	0.1%
LIBIDO INCREASED	-		1	0.1%
LIVER TENDERNESS	-		1	0.1%
LOSS OF CONSCIOUSNESS NEC	-		1	0.1%
LUNG CREPITATION	-		1	0.1%
MELAENA	-		1	0.1%
MEMORY IMPAIRMENT	-		1	0.1%
MENTAL IMPAIRMENT NOS	-		1	0.1%
MOOD SWINGS	-		1	0.1%
MUCOSAL DRYNESS NOS	-		1	0.1%
MUSCLE DISORDER NOS	-		1	0.1%
MUSCLE INJURY NOS	-		1	0.1%
NAIL INFECTION NOS	-		1	0.1%
NAIL REMOVAL NOS	-		1	0.1%
NASAL POLYPECTOMY	-		1	0.1%
NASAL POLYPS	-		1	0.1%
NECK STIFFNESS	-		1	0.1%
OBSTRUCTIVE AIRWAYS DISORDER NOS	-		1	0.1%
OESOPHAGITIS	-		1	0.1%
OESOPHAGITIS NOS	-		1	0.1%
ORAL FUNGAL INFECTION NOS	-		1	0.1%
ORAL PAIN	-		1	0.1%
PANIC ATTACK	-		1	0.1%
PERIORBITAL OEDEMA	-		1	0.1%
PERIPHERAL ISCHAEMIA NOS	-		1	0.1%
PHARYNGITIS STREPTOCOCCAL	-		1	0.1%
PHLEBITIS NOS	-		1	0.1%
PHOTOPHOBIA	-		1	0.1%
PLEURISY	-		1	0.1%
PLEURITIC PAIN	-		1	0.1%
PROCTALGIA	-		1	0.1%
PROSTATITIS	-		1	0.1%
PULSUS BIGEMINUS	-		1	0.1%
PUNCTURE SITE HAEMORRHAGE	-		1	0.1%
RASH ERYTHEMATOUS	-		1	0.1%
RED EYE	-		1	0.1%
RENAL PAIN	-		1	0.1%
RESPIRATORY DEPRESSION	-		1	0.1%

Note: only treatment-emergent AEs are included.
(Pag 5/8)

Appendix 12 Summary of adverse events in placebo-controlled studies, safety population (cont.)

Produced by sturlor on 17MAY01
Ro 47-0203, Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031
Table T31f: Summary of adverse events (including unrelated) by frequency
Population: Safety

Body system / Adverse event	Placebo N=288 No.	%	Bosentan N=677 No.	%
RESPIRATORY TRACT INFECTION VIRAL NOS	-		1	0.1%
RESTLESS LEG SYNDROME	-		1	0.1%
RETINAL DETACHMENT	-		1	0.1%
SENSATION OF HEAVINESS	-		1	0.1%
SEPSIS NOS	-		1	0.1%
SHIVERING	-		1	0.1%
SKIN DISCOLOURATION	-		1	0.1%
SPUTUM INCREASED	-		1	0.1%
STEVENS JOHNSON SYNDROME	-		1	0.1%
SUBARACHNOID HAEMORRHAGE	-		1	0.1%
SUDDEN DEATH UNEXPLAINED	-		1	0.1%
TASTE DISTURBANCE	-		1	0.1%
TEMPOROMANDIBULAR JOINT ARTHRALGIA	-		1	0.1%
TENDERNESS NOS	-		1	0.1%
THIRST	-		1	0.1%
TIRED EYES	-		1	0.1%
TONGUE DISCOLOURATION NOS	-		1	0.1%
TONGUE DRY	-		1	0.1%
TOOTH EXTRACTION NOS	-		1	0.1%
TOOTH INFECTION	-		1	0.1%
TRAUMATIC CHEST INJURY NOS	-		1	0.1%
TUNNEL VISION	-		1	0.1%
UPPER RESPIRATORY TRACT INFECTION VIRAL NOS	-		1	0.1%
URINARY FREQUENCY	-		1	0.1%
URINARY TRACT INJURY NOS	-		1	0.1%
URINE DISCOLOURATION	-		1	0.1%
VENOUS THROMBOSIS NOS	-		1	0.1%
VENTRICULAR ARRHYTHMIA NOS	-		1	0.1%
VISUAL ACUITY REDUCED	-		1	0.1%
WHITE BLOOD CELL INCREASED	-		1	0.1%
XEROPHTHALMIA	-		1	0.1%
PLEURAL EFFUSION	3	1.0%	-	
ABNORMAL DREAMS	2	0.7%	-	
ATRIAL FLUTTER	2	0.7%	-	
CHRONIC OBSTRUCTIVE AIRWAYS DISEASE	2	0.7%	-	
GASTRIC EROSIONS	2	0.7%	-	
HAEMATOMA NOS	2	0.7%	-	
HEPATOMEGALY	2	0.7%	-	
HOARSENESS	2	0.7%	-	
INJECTION SITE INFECTION	2	0.7%	-	
PHARYNGITIS NOS	2	0.7%	-	
SCRATCH	2	0.7%	-	
SYSTEMIC LUPUS ERYTHEMATOSUS SYNDROME AGGRAVATED	2	0.7%	-	
WEIGHT INCREASED	2	0.7%	-	
ABRASION NOS	1	0.3%	-	
ABSCESS NOS	1	0.3%	-	
ACUTE ABDOMEN	1	0.3%	-	
AGITATION	1	0.3%	-	
APNOEA	1	0.3%	-	
ARTHRITIS NOS	1	0.3%	-	
ASTHMA AGGRAVATED	1	0.3%	-	
BLEEDING VARICOSE VEIN	1	0.3%	-	
BLINDNESS NEC	1	0.3%	-	
BLOOD ALBUMIN DECREASED	1	0.3%	-	
BLOOD BILIRUBIN INCREASED	1	0.3%	-	
CANDIDAL INFECTION NOS	1	0.3%	-	
CERVICAL ROOT PAIN	1	0.3%	-	

Note: only treatment-emergent AEs are included.
(Pag 6/8)

Appendix 12 Summary of adverse events in placebo-controlled studies, safety population (cont.)

Produced by sturlor on 17MAY01

Ro 47-0203, Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031
Table T31f: Summary of adverse events (including unrelated) by frequency
Population: Safety

Body system / Adverse event	Placebo N=288 No.	Placebo %	Bosentan N=677 No.	Bosentan %
CHOLELITHIASIS	1	0.3%	-	
COAGULATION DISORDER NOS	1	0.3%	-	
COLITIS NOS	1	0.3%	-	
COLLAPSE	1	0.3%	-	
COLONOSCOPY	1	0.3%	-	
CONGESTIVE (DILATED) CARDIOMYOPATHY	1	0.3%	-	
CRANIOTOMY	1	0.3%	-	
CYANOSIS NOS	1	0.3%	-	
DEPRESSION AGGRAVATED	1	0.3%	-	
DRUG TOXICITY NOS	1	0.3%	-	
DRY EYE NEC	1	0.3%	-	
DYSPNOEA PAROXYSMAL NOCTURNAL	1	0.3%	-	
EXTRASYSTOLES NOS	1	0.3%	-	
EYE HAEMORRHAGE NOS	1	0.3%	-	
FLUID OVERLOAD	1	0.3%	-	
FOOT ULCER	1	0.3%	-	
GASTRIC POLYPS	1	0.3%	-	
HALLUCINATION NOS	1	0.3%	-	
HAND FRACTURE	1	0.3%	-	
HEPATIC NEOPLASM NOS	1	0.3%	-	
HIATUS HERNIA	1	0.3%	-	
HYPERKALAEMIA	1	0.3%	-	
HYPERLIPIDAEMIA NOS	1	0.3%	-	
HYPERSOMNIA	1	0.3%	-	
HYPERTHYROIDISM	1	0.3%	-	
HYPOTHERMIA	1	0.3%	-	
HYPOTHYROIDISM	1	0.3%	-	
INTESTINAL STOMA SITE BLEEDING	1	0.3%	-	
JOINT SPRAIN	1	0.3%	-	
KERATOACANTHOMA	1	0.3%	-	
LACRIMATION ABNORMAL NOS	1	0.3%	-	
LARYNGITIS NOS	1	0.3%	-	
LEG ULCER (EXC VARICOSE)	1	0.3%	-	
LEUCOCYTOSIS NOS	1	0.3%	-	
LEUKOPENIA NOS	1	0.3%	-	
LUNG CANCER STAGE UNSPECIFIED (EXC METASTATIC TUMOURS TO LUNG)	1	0.3%	-	
LYMPHOPENIA	1	0.3%	-	
MALAISE	1	0.3%	-	
MIXED CONNECTIVE TISSUE DISEASE	1	0.3%	-	
OEDEMA UPPER LIMB	1	0.3%	-	
PERIPHERAL COLDNESS	1	0.3%	-	
PERIPHERAL SWELLING	1	0.3%	-	
PERONEAL NERVE PALSY	1	0.3%	-	
PERSONALITY DISORDER NOS	1	0.3%	-	
PETECHIAE	1	0.3%	-	
PHARYNGITIS VIRAL NOS	1	0.3%	-	
PHOTOPSIA	1	0.3%	-	
PHOTOSENSITIVE RASH	1	0.3%	-	
PILONIDAL SINUS INFECTED	1	0.3%	-	
PLANTAR FASCIITIS	1	0.3%	-	
PORTAL HYPERTENSION	1	0.3%	-	
POSTNASAL DRIP	1	0.3%	-	
PROSTATIC PAIN	1	0.3%	-	
PROTEINURIA PRESENT	1	0.3%	-	
PULMONARY ARTERIAL WEDGE PRESSURE INCREASED	1	0.3%	-	
PULMONARY EMBOLISM	1	0.3%	-	
RASH MACULO-PAPULAR	1	0.3%	-	

Note: only treatment-emergent AEs are included.
(Pag 7/8)

Appendix 12 Summary of adverse events in placebo-controlled studies, safety population (cont.)

Produced by sturlor on 17MAY01
Ro 47-0203, Protocols: AC-52351 AC-52352 BC-15064(II) BD-14884 NC-15018 NC-15020 NC-15462 NN-15031
Table T31f: Summary of adverse events (including unrelated) by frequency
Population: Safety

Body system / Adverse event	Placebo N=288 No.	%	Bosentan N=677 No.	%
RASH PAPULAR	1	0.3%	-	
RESPIRATORY ARREST (EXC NEONATAL)	1	0.3%	-	
RESPIRATORY DISORDER NOS	1	0.3%	-	
RETINOPATHY DIABETIC	1	0.3%	-	
RIB FRACTURE	1	0.3%	-	
RIGHT VENTRICULAR FAILURE	1	0.3%	-	
SINUS BRADYCARDIA	1	0.3%	-	
SINUS TACHYCARDIA	1	0.3%	-	
SKIN CARCINOMA NOS	1	0.3%	-	
SKIN INJURY NOS	1	0.3%	-	
SKIN OPERATION NOS	1	0.3%	-	
SMALL INTESTINAL PERFORATION NOS	1	0.3%	-	
SPLENOMEGALY	1	0.3%	-	
SQUAMOUS CELL CARCINOMA	1	0.3%	-	
STOMATITIS	1	0.3%	-	
SUPRAVENTRICULAR TACHYCARDIA	1	0.3%	-	
TENDONITIS	1	0.3%	-	
THERAPEUTIC AGENT TOXICITY	1	0.3%	-	
THROMBOPHLEBITIS DEEP	1	0.3%	-	
THROMBOSIS NOS	1	0.3%	-	
TOOTH REPAIR	1	0.3%	-	
TRACHEOBRONCHITIS	1	0.3%	-	
URINARY RETENTION	1	0.3%	-	
VAGINAL CANDIDIASIS	1	0.3%	-	
VAGINAL HAEMORRHAGE	1	0.3%	-	
VOCAL CORD PARALYSIS	1	0.3%	-	
WOUND INFECTION NEC	1	0.3%	-	

Note: only treatment-emergent AEs are included.
(Pag 8/8)

Appendix 13 Most frequent adverse events (≥ 3%) in placebo-controlled studies at the doses evaluated in pulmonary arterial hypertension (250–500 mg/day), safety population

Produced by sturlor on 17MAY01

Ro 47-0203, Protocols: AC-52351 AC-52352 NC-15020
Table T31b_3: Summary of adverse events (including unrelated) by frequency
Population: Safety

Only events >= 3% and occurring for more than 3 patients (Bosentan)

Body system / Adverse event	Placebo N=129 No.	Placebo N=129 %	Bosentan 250-500 mg/d N=214 No.	Bosentan 250-500 mg/d N=214 %
ALL BODY SYSTEMS				
Total pts with at least one AE	103	79.8%	180	84.1%
Total number of AEs	368		622	
HEADACHE NOS	26	20.2%	43	20.1%
FLUSHING	4	3.1%	21	9.8%
UPPER RESPIRATORY TRACT INFECTION NOS	10	7.8%	20	9.3%
DIZZINESS (EXC VERTIGO)	15	11.6%	18	8.4%
NASOPHARYNGITIS	6	4.7%	18	8.4%
NAUSEA	11	8.5%	14	6.5%
SYNCOPE	7	5.4%	14	6.5%
OEDEMA LOWER LIMB	4	3.1%	14	6.5%
HEPATIC FUNCTION ABNORMAL NOS	2	1.6%	14	6.5%
DIARRHOEA NOS	6	4.7%	13	6.1%
COUGH	9	7.0%	12	5.6%
BRONCHITIS NOS	8	6.2%	12	5.6%
HYPOTENSION NOS	3	2.3%	11	5.1%
PULMONARY HYPERTENSION NOS AGGRAVATED	16	12.4%	10	4.7%
ARTHRALGIA	6	4.7%	10	4.7%
PALPITATIONS	4	3.1%	10	4.7%
OEDEMA NOS	2	1.6%	10	4.7%
DYSPNOEA NOS	9	7.0%	9	4.2%
CHEST PAIN NEC	7	5.4%	9	4.2%
SINUSITIS NOS	5	3.9%	9	4.2%
EPISTAXIS	5	3.9%	8	3.7%
RESPIRATORY TRACT INFECTION NOS	4	3.1%	8	3.7%
DYSPEPSIA	1	0.8%	8	3.7%
INFLUENZA	11	8.5%	7	3.3%
ABDOMINAL PAIN NOS	8	6.2%	7	3.3%
BACK PAIN	4	3.1%	7	3.3%

Note: only treatment-emergent AEs are included.
Only AEs with an incidence >3% before rounding are included.
(Pag 1/1)

Appendix 14 Sponsor-defined critical values for laboratory parameters

Parameter	Standard Unit	Sponsor's Reference Range	Marked Reference Range	Significant Shift From Baseline
HEMATOLOGY				
Hemoglobin	g/dl	M: 13-18 F: 12-16	11-20	15%
Hematocrit	Fraction	M: 0.42-0.52 F: 0.37-0.48	0.36-0.60	15%
Erythrocytes	10E12/l	4.2-5.9	3.2-5.6	15%
Leucocytes (total)	10E9/l	4.3-10.8	3-18	30%
Neutrophils	10E9/l	1.83-7.25	1.50 or more	Decrease 20%
Eosinophils	10E9/l	0-0.7	0-1.5	Increase 100%
Platelets	10E9/l	150-450	100-700	Decrease 30% Increase 50%
BIOCHEMISTRY				
AST	U/l	0-25	0-50	Increase 50%
ALT	U/l	0-30	0-60	Increase 50%
Gamma-glutamyl transferase	U/l	0-60	0-120	Increase 50%
Bilirubin total	umol/l	0-17.1	0-34.2	Increase 50%
Glucose fasting	mmol/l	3.9-6.1	2.8-11.1	75%
Creatinine	umol/l	0-133	0-154	Increase 75%
Sodium	mmol/l	133-145	130-150	7%
Potassium	mmol/l	3.5-5.0	3.0-6.0	Decrease 10% Increase 20%
Chloride	mmol/l	100-108	95-115	7%
Albumin	g/l	35-55	27-58	10%
Alkaline phosphatase	U/l	0-100	0-190	Increase 50%
BUN / urea	mmol/l	2.9-8.9	0-14.3	Increase 75%
Cholesterol	mmol/l	0-6.2	0-8.3	Increase 50%
Triglycerides	mmol/l	0.45-1.70	0-2.83	Increase 100%
Protein total	g/l	60-80	55-87	20%

ALT = alanine aminotransferases, AST = aspartate aminotransferases, BUN = blood urea nitrogen.

Appendix 15 Summary of treatment-emergent ECG findings in placebo-controlled studies in pulmonary arterial hypertension (AC-052-351 and AC-052-352), safety population

Produced by madesu on 27JUN01

Ro 47-0203, Protocols: AC-52351 AC-52352
Table T65f_12: Summary of treatment-emergent ECG findings
Population: Safety

ECG finding	Bosentan 250 mg/d N=95 No.	%	Bosentan 500 mg/d N=70 No.	%	Bosentan (all) N=165 No.	%	Placebo N=80 No.	%
Total pts with at least one ECG	40	42.1%	25	35.7%	65	39.4%	36	45.0%
Total number of ECGs	72		34		106		57	
ST-T CHANGES	12	12.6%	4	5.7%	16	9.7%	8	10.0%
RIGHT AXIS DEVIATION	9	9.5%	4	5.7%	13	7.9%	9	11.3%
INTRA-VENTRICULAR CONDUCTION DEFECT	5	5.3%	5	7.1%	10	6.1%	3	3.8%
RIGHT VENTRICULAR HYPERTROPHY	8	8.4%	1	1.4%	9	5.5%	5	6.3%
RIGHT ATRIAL ENLARGEMENT	6	6.3%	-		6	3.6%	6	7.5%
VENTRICULAR EXTRASYSTOLES	3	3.2%	3	4.3%	6	3.6%	2	2.5%
NON SPECIFIC ST-T CHANGES	6	6.3%	-		6	3.6%	1	1.3%
ATRIAL FLUTTER AND / OR FIBRILLATION	3	3.2%	3	4.3%	6	3.6%	-	
LEFT ATRIAL ENLARGEMENT	3	3.2%	3	4.3%	6	3.6%	-	
ATRIOVENTRICULAR BLOCK FIRST DEGREE	4	4.2%	1	1.4%	5	3.0%	3	3.8%
OTHER FINDINGS	1	1.1%	3	4.3%	4	2.4%	-	
SINUS BRADYCARDIA	3	3.2%	-		3	1.8%	3	3.8%
SINUS TACHYCARDIA	1	1.1%	2	2.9%	3	1.8%	2	2.5%
BILATERAL ATRIAL ENLARGEMENT	3	3.2%	-		3	1.8%	-	
SINUS ARRHYTHMIA	-		2	2.9%	2	1.2%	1	1.3%
HIGH VOLTAGE	1	1.1%	-		1	0.6%	2	2.5%
ATRIOVENTRICULAR BLOCK SECOND DEGREE MOBITZ I	-		1	1.4%	1	0.6%	1	1.3%
BUNDLE BRANCH BLOCK RIGHT	1	1.1%	-		1	0.6%	1	1.3%
EVIDENCE OF MYOCARDIAL INFARCTION	1	1.1%	-		1	0.6%	1	1.3%
LOW VOLTAGE	1	1.1%	-		1	0.6%	1	1.3%
SUPRAVENTRICULAR EXTRASYSTOLES	-		1	1.4%	1	0.6%	1	1.3%
LEFT VENTRICULAR HYPERTROPHY	1	1.1%	-		1	0.6%	-	
PACEMAKER RHYTHM	-		1	1.4%	1	0.6%	-	
PROLONGED QT	-		-		-		4	5.0%
BUNDLE BRANCH BLOCK BILATERAL	-		-		-		1	1.3%
LEFT AXIS DEVIATION	-		-		-		1	1.3%
LEFT POSTERIOR HEMI-BLOCK	-		-		-		1	1.3%

(Pag 1/1)

Appendix 16 Summary of mean changes from baseline to study end in quantitative 12-lead ECG parameters in studies in pulmonary arterial hypertension (AC-052-351 and AC-052-352), safety population

Produced by sturlor on 18AUG00, condensed manchek 2JULY01

Ro 47-0203, Protocol: AC-052-351
Table T42b (modified): Change to study end in ECG (12 lead)
Population: ITT

	Bosentan	Placebo
	N=21	N=11
PQ (PR) (ms)		
n	20	10
Baseline	174.4 ± 26.6	166.3 ± 21.7
Study end	174.8 ± 27.9	166.9 ± 22.9
Absolute change	0.4 ± 10.4	0.6 ± 15.5
Percent change	0.2 ± 5.8	0.7 ± 9.1
QRS (ms)		
n	20	10
Baseline	93.8 ± 12.9	91.1 ± 14.9
Study end	91.6 ± 12.5	89.5 ± 10.8
Absolute change	-2.2 ± 6.7	-1.6 ± 8.3
Percent change	-2.0 ± 7.2	-0.8 ± 9.3
QT (ms)		
n	20	10
Baseline	381.5 ± 30.8	353.7 ± 42.6
Study end	382.1 ± 34.4	356.6 ± 28.3
Absolute change	0.7 ± 28.1	2.9 ± 25.0
Percent change	0.3 ± 7.1	1.4 ± 7.5
HEART RATE (bpm)		
n	20	10
Baseline	80.2 ± 9.7	86.7 ± 11.6
Study end	79.1 ± 11.8	89.9 ± 9.7
Absolute change	-1.2 ± 13.0	3.2 ± 7.0
Percent change	-0.6 ± 17.2	4.3 ± 8.1

Note: Study end value is last valid value after baseline
Values are mean ± standard deviation.

Appendix 16 Summary of mean changes from baseline to study end in quantitative 12-lead ECG parameters in studies in pulmonary arterial hypertension (AC-052-351 and AC-052-352), safety population (cont.)

Produced by madesu on 04MAY01

Ro 47-0203, Protocol: AC-052-352
Table T30: Mean change from baseline up to end of study in 12-lead ECG data
Population: Safety

	Bosentan 125 mg N=74	Bosentan 250 mg N=70	All Bosentan N=144	Placebo N=69
PQ (PR) Interval (ms)				
n	65	67	132	66
Baseline	166.3 ± 23.7	172.7 ± 33.4	169.5 ± 29.1	169.5 ± 29.1
Last up to end of study	167.4 ± 25.1	170.2 ± 42.5	168.8 ± 34.9	171.3 ± 34.4
Change from baseline	1.1 ± 22.2	-2.5 ± 37.2	-0.7 ± 30.7	1.8 ± 22.1
% Change from baseline	1.5 ± 14.6	-0.5 ± 19.2	0.5 ± 17.1	1.6 ± 13.3
QRS Interval (ms)				
n	73	68	141	67
Baseline	93.0 ± 20.6	94.3 ± 20.3	93.6 ± 20.4	90.1 ± 15.6
Last up to end of study	91.8 ± 20.6	94.6 ± 20.5	93.2 ± 20.6	92.4 ± 14.9
Change from baseline	-1.2 ± 11.8	0.3 ± 13.9	-0.5 ± 12.8	2.4 ± 15.0
% Change from baseline	-0.4 ± 12.9	1.8 ± 18.1	0.7 ± 15.6	5.2 ± 27.4
QT Interval (ms)				
n	73	68	141	67
Baseline	384.5 ± 43.2	375.3 ± 45.9	380.0 ± 44.6	377.0 ± 44.0
Last up to end of study	386.1 ± 42.4	373.7 ± 47.8	380.1 ± 45.4	375.6 ± 44.1
Change from baseline	1.7 ± 40.1	-1.6 ± 45.1	0.1 ± 42.5	-1.4 ± 37.8
% Change from baseline	1.0 ± 11.1	0.3 ± 13.0	0.7 ± 12.0	0.1 ± 10.3
Heart Rate (bpm)				
n	73	68	141	67
Baseline	80.2 ± 13.0	83.0 ± 16.2	81.5 ± 14.6	79.9 ± 12.0
Last up to end of study	77.5 ± 13.6	82.0 ± 14.2	79.7 ± 14.0	81.7 ± 14.0
Change from baseline	-2.7 ± 10.4	-0.9 ± 12.9	-1.8 ± 11.6	1.8 ± 11.5
% Change from baseline	-2.8 ± 12.5	0.7 ± 18.3	-1.1 ± 15.6	2.9 ± 14.6

Note: Values are mean ± standard deviation.

Appendix 17 In-depth analysis of the incidence of ALT and/or AST > 3 × ULN in all placebo-controlled studies, safety population

ALT > 3 x Upper normal or AST > 3 x Upper normal — 17MAY01

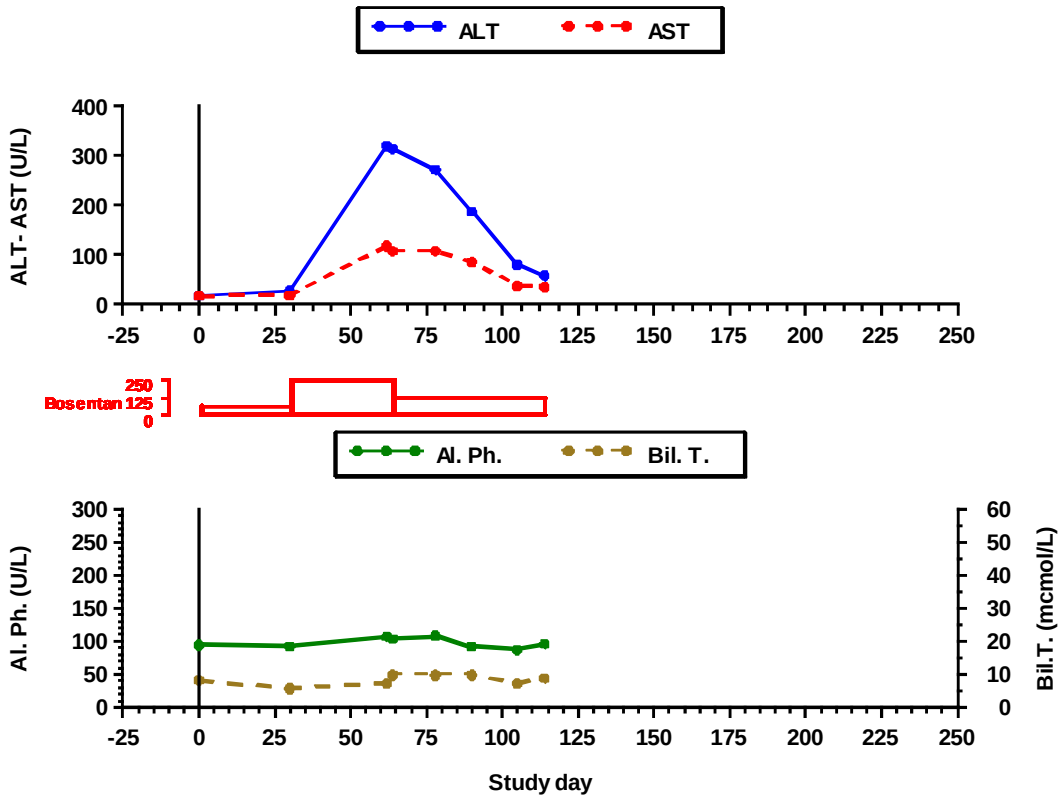
		100 B	100 P	250-500 B	250-500 P	1000-1500 B	1000-1500 P	2000 B	2000 P	All B	All P
PAH	2-12W							0.0% 0/ 4 0.0%	0.0% 0/ 1 0.0%	0.0% 0/ 4 0.0%	0.0% 0/ 1 0.0%
	> 12W			12.7% 21/165 0.0% 0/ 79 +12.7% **						12.7% 21/165 0.0% 0/ 79 +12.7% **	
	All									12.4% 21/169 0.0% 0/ 80 +12.4% **	
CHF	2-12W							4.2% 2/ 48 0.0%	0/ 21 +4.2%	4.2% 2/ 48 0.0%	0/ 21 +4.2%
	> 12W					15.8% 37/234 2.4% 3/124 +13.4% **				15.8% 37/234 2.4% 3/124 +13.4% **	
	All									13.8% 39/282 2.1% 3/145 +11.8% **	
HTN	2-12W	2.1% 1/ 48 2.1% -0.0%	1/ 47	4.3% 2/ 46 2.1% +2.2%	1/ 47	11.4% 5/ 44 2.1% +9.2%	1/ 47	10.0% 5/ 50 2.1% +7.9%	1/ 47	6.9% 13/188 2.1% +4.8%	1/ 47
	> 12W										
	All										
SAH	2-12W					5.3% 1/ 19 12.5% -7.2%	1/ 8			5.3% 1/ 19 12.5% -7.2%	1/ 8
	> 12W										
	All										
All	2-12W	2.1% 1/ 48 2.1% -0.0%	1/ 47	4.3% 2/ 46 2.1% +2.2%	1/ 47	9.5% 6/ 63 3.6% +5.9%	2/ 55	6.9% 7/102 1.4% +5.4%	1/ 69	6.2% 16/259 2.6% +3.6%	2/ 77
	> 12W			12.7% 21/165 0.0% 0/ 79 +12.7% **		15.8% 37/234 2.4% 3/124 +13.4% **				14.5% 58/399 1.5% 3/203 +13.1% **	
	All			10.9% 23/211 0.8% 1/126 +10.1% **		14.5% 43/297 2.8% 5/179 +11.7% **				11.2% 74/658 1.8% 5/280 +9.5% **	

Fisher's exact test # : p <0.10 * : p <0.05 **: p <0.01
The duration 'All' data are reported only when there are 2 elements in the cell

Appendix 18 Patient with transient increase in liver aminotransferases with complete resolution after dose reduction, Study AC-052-352 in pulmonary arterial hypertension

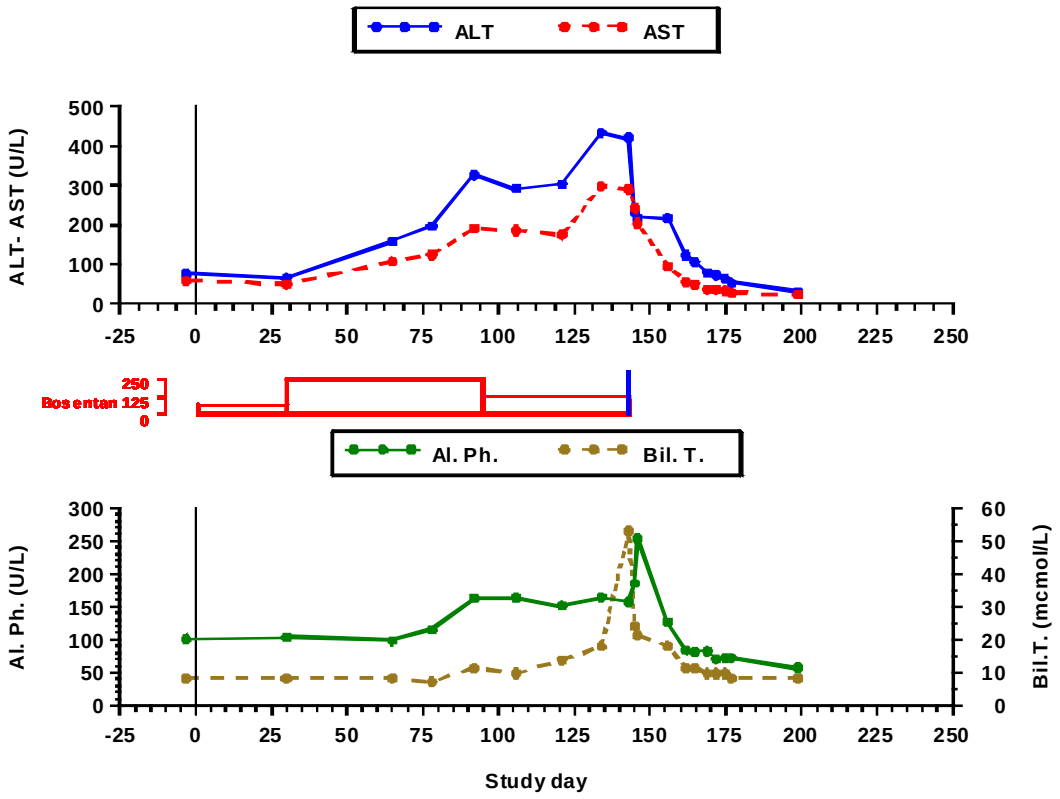
Patient 208 20007 (bosentan 250 mg b.i.d.): A 57-year-old white female with pulmonary arterial hypertension due to primary pulmonary hypertension. The patient experienced an asymptomatic increase in ALT (> 8 × ULN). The dose was cut by half (to 125 mg b.i.d.) on Day 63, and as a result, the ALT levels started dropping and returned to baseline levels at the end of Period 1. The patient was rolled over to the open-label study (AC-052-354).

Breathe-1 - Patient No. 208/20007-Bosentan 250 mg



Appendix 19 Patient with gradual onset of increased liver aminotransferases associated with increased bilirubin > 3 × ULN and rapid resolution after stopping treatment, Study AC-052-352 in pulmonary arterial hypertension

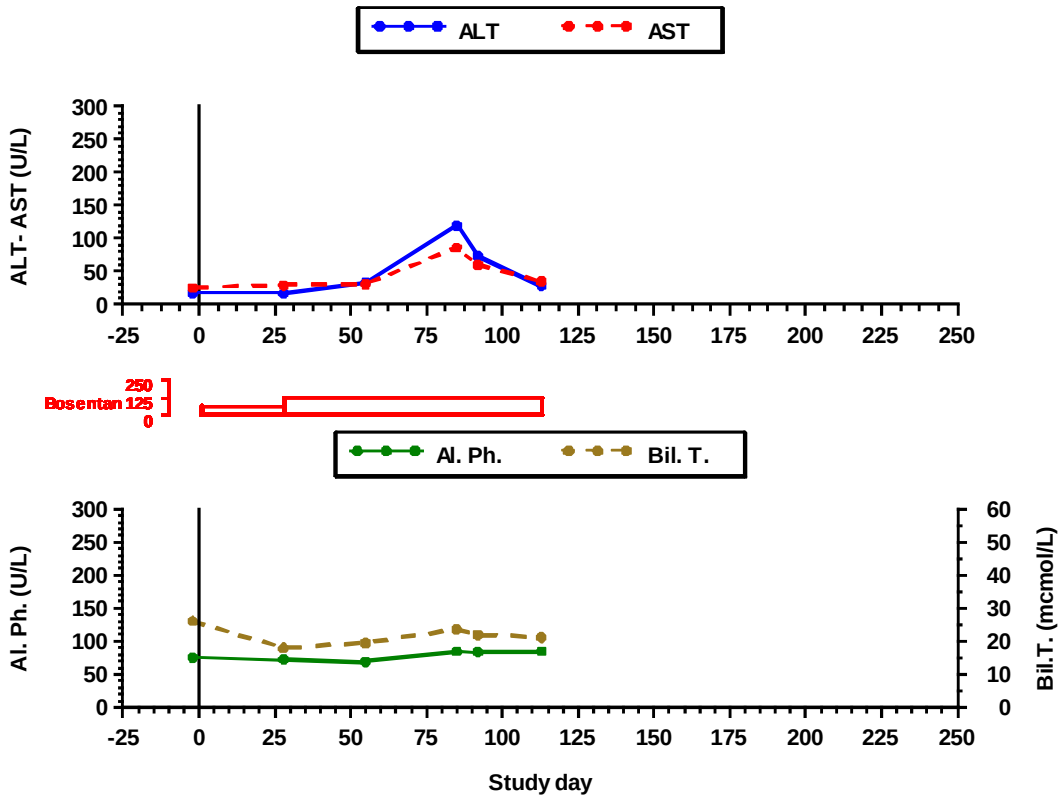
Patient 106 10008 (bosentan 250 mg b.i.d.): A 53-year-old black female with pulmonary arterial hypertension due to mixed connective tissue disease. The patient experienced asymptomatic increases in liver aminotransferases (> 8 × ULN), alkaline phosphatase, and bilirubin. Stopping treatment (Day 143) resulted in a rapid return of the liver parameters to baseline levels. See detailed narrative Section 8.2.3.



Appendix 20 Patient with transient increase in liver aminotransferases with complete resolution while continuing treatment at the target dose, Study AC-052-352 in pulmonary arterial hypertension

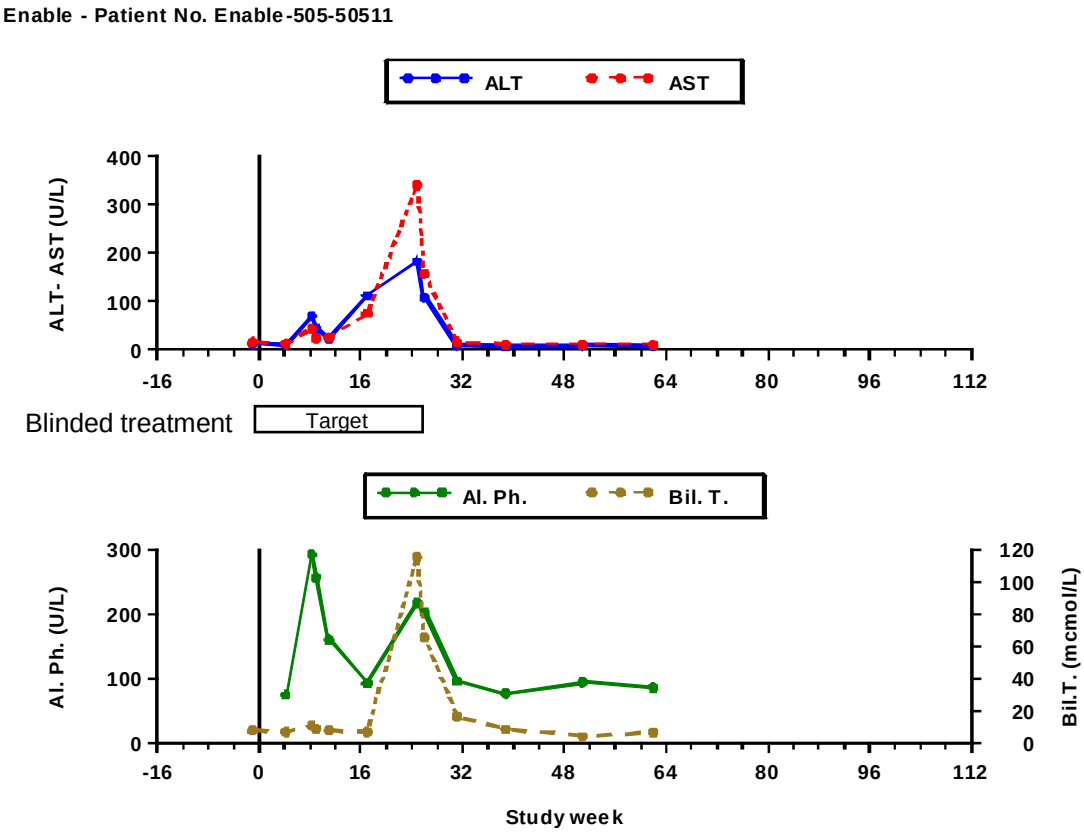
Patient 109 10065 (bosentan 125 mg b.i.d.): A 54-year-old white male with pulmonary arterial hypertension due to primary pulmonary hypertension. The patient experienced an asymptomatic increase in liver aminotransferases (> 3 × ULN and < 5× ULN). Treatment remained stable during the trial. The liver aminotransferases returned to baseline levels, and the patient was rolled over to the open-label study (AC-052-364).

Breathe-1 - Patient No. 109/10065-Bosentan 125 mg



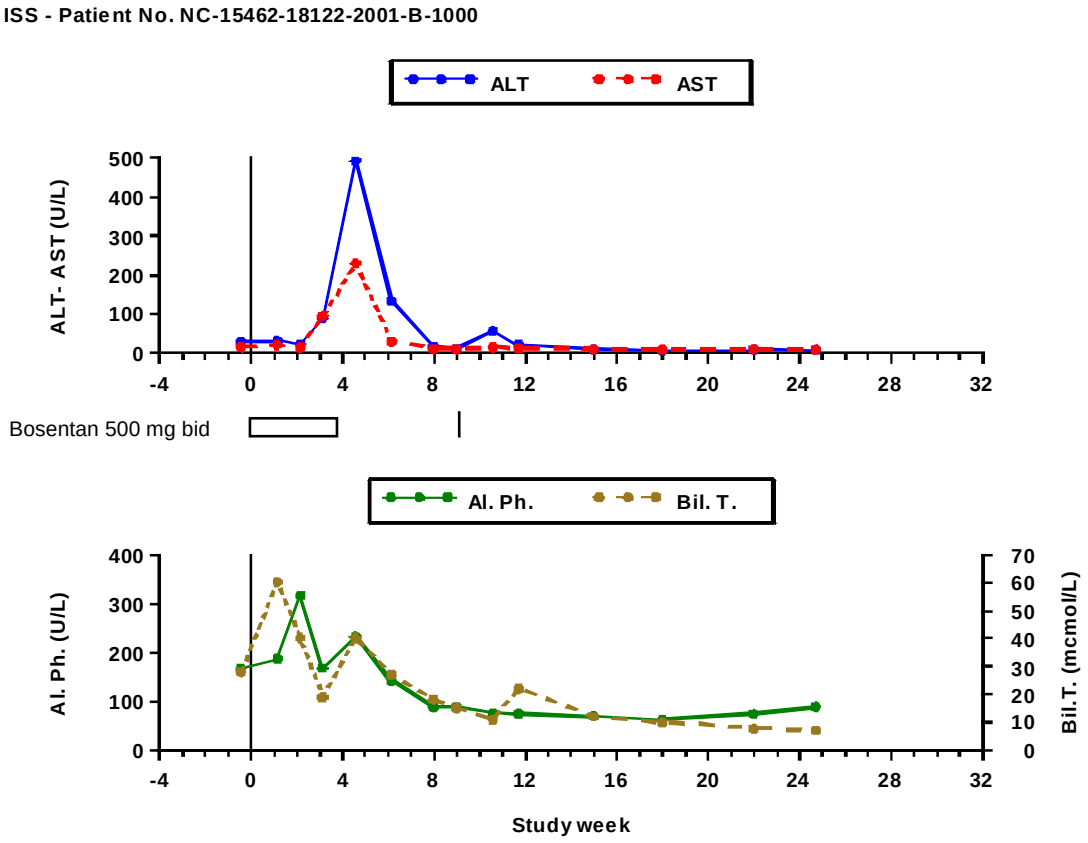
Appendix 21 Patient with ALT and bilirubin >3 × ULN, ENABLE in chronic heart failure

Patient 50511 (blinded treatment): Treatment discontinued in Week 26. See narrative Section 8.2.3.



Appendix 22 Patients in Study NC15462 with a large increase in ALT(> 15 × ULN)

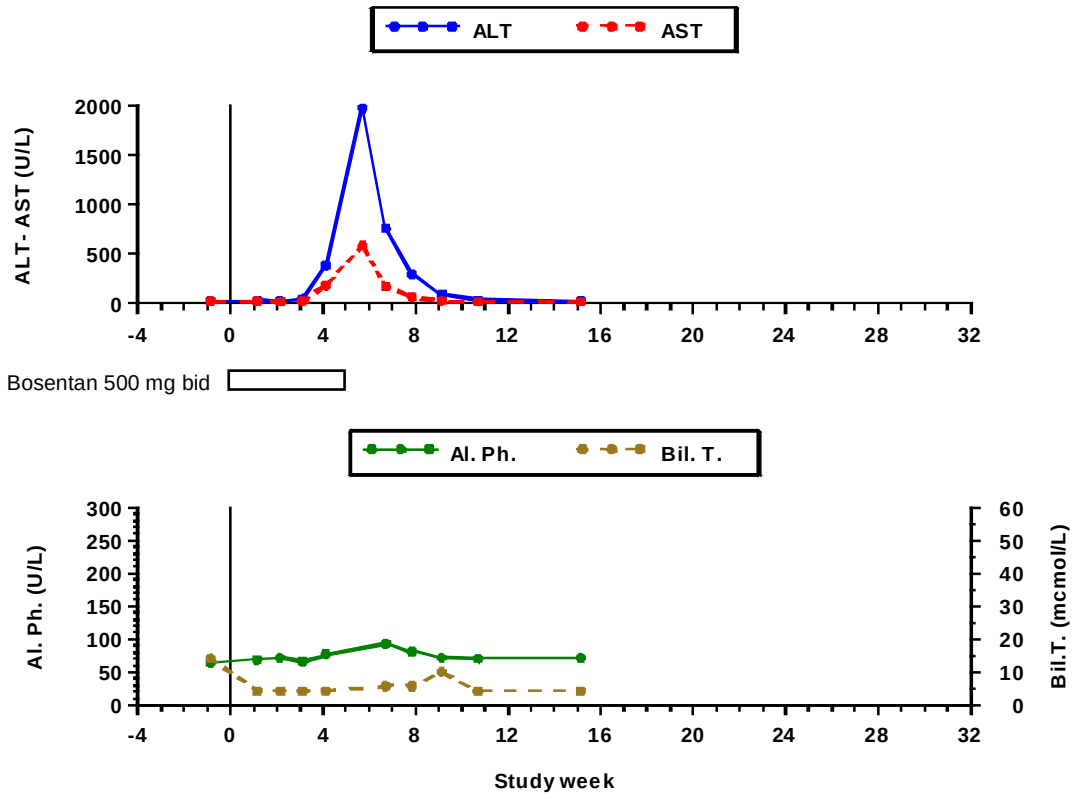
Patient 18122/2001 (bosentan 500 mg b.i.d.): This 67-year-old white male with severe CHF (NYHA Class IIIb, ejection fraction 25%) was hospitalized 8 days after randomization due to pneumonia and worsening heart failure. He was treated with diuretics and i.v. ampicillin and ceftriaxone, which was changed from ceftriaxon to imipenem because of ongoing fever. Paracetamol was given PRN from Days 21 to 25. On Day 22 an asymptomatic elevation of liver enzymes was noted associated with an increase in alkaline phosphatase, bilirubin and bile acids, which were already above the ULN at baseline (from 168 to 233 U/l, from 28.2 to 40.2 µmol/l and from 22.2 to 69.9 µmol/l respectively). Bosentan was interrupted on Day 27 (Week 4) and the patient was discharged in improved condition on Day 39. Re-introduction of bosentan on Day 63 (Week 9) was associated with fever and chills. Study medication was permanently stopped, and fever resolved the following day. Eosinophil count was not increased during the trial.



Appendix 22 Patients in Study NC15462 with a large increase in ALT(> 15 × ULN) (cont.)

Patient 18164/6331 (bosentan 500 mg b.i.d.): This 44-year-old black male with severe CHF (NYHA Class IIIb and ejection fraction 15%) was diagnosed with diabetes mellitus type II on Day 8, and glibenclamide (glyburide) was initiated on that day. The patient also experienced two episodes of hypotension of moderate intensity on Days 12 and 22 and one episode of headache, for which he received paracetamol, on Day 26. Transaminase levels were found to be elevated on Day 29 (Week 4), reaching the highest level on Day 40. Alkaline phosphatase and bilirubin remained within normal limits. Bosentan was stopped on Day 35 (Week 5), as was glibenclamide. Aminotransferases decreased to < 2 × ULN on Day 75. The patient was hospitalized on several occasions thereafter for various reasons (worsening heart failure, evaluation for heart transplant, bradycardia suspected to be due to digoxin and pyrexia). The transaminase levels remained within normal limits until completion of the study on Day 176. Eosinophil count remained unchanged during the trial.

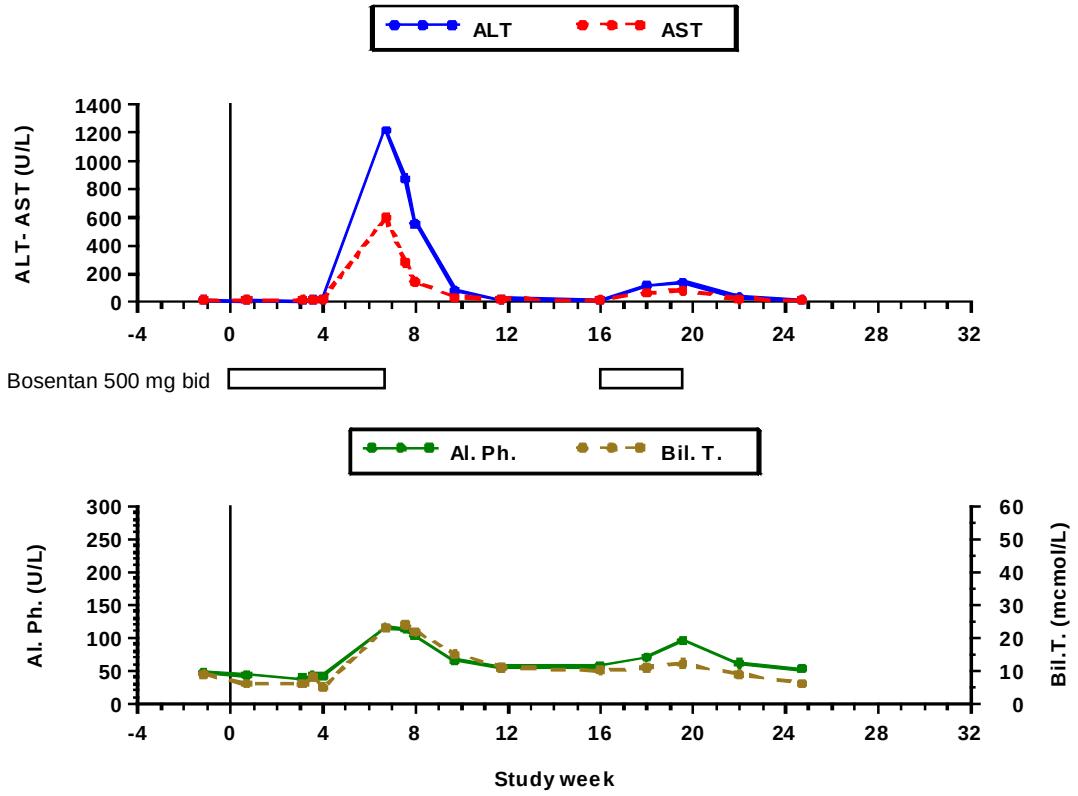
ISS - Patient No. NC-15462-18164-6331-B-1000



Appendix 22 Patients in Study NC15462 with a large increase in ALT(> 15 × ULN) (cont.)

Patient 18127/8082 (bosentan 500 mg b.i.d): This 71-year-old white female with severe CHF (NYHA class IIIb and ejection fraction 25%) had an asymptomatic increase in liver transaminase levels on Day 47 (Week 7). Alkaline phosphatase and bilirubin increased slightly. Study medication was stopped on that day, and the aminotransferases returned to normal levels. Study medication was reintroduced on Day 112 (Week 16) and permanently stopped on Day 136 (Week 19-20) due to recurrence of the increase in intransaminase levels. Eosinophil count remained within normal limits during the trial.

ISS - Patient No. NC-15462-18127-8082-B-1000



Appendix 23 Proposed guidelines in case of ALT/AST elevations

ALT/AST levels	Treatment and monitoring recommendations
> 3 and ≤ 5 × ULN	Confirm by another liver test; if confirmed, reduce the daily dose or interrupt treatment, monitor aminotransferase levels at least every 2 weeks. If the aminotransferase levels return to pre-treatment values, continue or re-introduce the treatment as appropriate (see below).
≥ 5 and < 8 × ULN	Confirm by another liver test; if confirmed, stop treatment and monitor aminotransferase levels at least every 2 weeks. Once the aminotransferase levels return to pre-treatment values consider re-introduction of the treatment (see below).
≥ 8 × ULN	Treatment should be stopped and re-introduction of bosentan should not be considered.

In case of clinical symptoms of liver injury, treatment should be stopped and re-introduction of bosentan should not be considered.

Re-introduction of treatment with bosentan should only be considered if the potential benefits of treatment with bosentan outweigh the potential risks and when aminotransferase levels are within pre-treatment values. Aminotransferase levels should then be checked within 3 days after re-introduction and thereafter, according to the recommendations above.

Appendix 24 In-depth summary of mean changes in hemoglobin concentration from baseline to end of treatment in placebo-controlled studies, safety population

Mean change from baseline for Hemoglobin g/dL - change from BL to last value up to end of treatment + 1 day 22MAY01

		100		250-500		1000-1500		2000		All	
		B	P	B	P	B	P	B	P	B	P
PAH	2-12W							-2.25 N=4 -3.88	1.63 N=1	-2.25 N=4 -3.88	1.63 N=1
	> 12W			-0.96 N=161 -0.96 **	0.01 N=79					-0.96 N=161 -0.96 **	0.01 N=79
	All									-0.99 N=165 -1.02	0.03 N=80
CHF	2-12W							-1.42 N=43 -0.78 **	-0.64 N=20	-1.42 N=43 -0.78 **	-0.64 N=20
	> 12W					-1.06 N=208 -0.93 **	-0.13 N=116			-1.06 N=208 -0.93 **	-0.13 N=116
	All									-1.12 N=251 -0.91	-0.21 N=136
HTN	2-12W	-0.39 N=48 -0.31 #	-0.08 N=46	-0.37 N=45 -0.29 #	-0.08 N=46	-0.37 N=44 -0.28 #	-0.08 N=46	-0.82 N=48 -0.74 **	-0.08 N=46	-0.49 N=185 -0.41 **	-0.08 N=46
	> 12W										
	All										
SAH	2-12W					-1.73 N=17 -0.69	-1.04 N=7			-1.73 N=17 -0.69	-1.04 N=7
	> 12W										
	All										
All	2-12W	-0.39 N=48 -0.31	-0.08 N=46	-0.37 N=45 -0.29	-0.08 N=46	-0.74 N=61 -0.54	-0.21 N=53	-1.16 N=95 -0.93	-0.22 N=67	-0.77 N=249 -0.47	-0.30 N=74
	> 12W			-0.96 N=161 -0.96	0.01 N=79	-1.06 N=208 -0.93	-0.13 N=116			-1.02 N=369 -0.94	-0.08 N=195
	All			-0.83 N=206 -0.80	-0.03 N=125	-0.99 N=269 -0.83	-0.16 N=169			-0.92 N=618 -0.78	-0.14 N=269

Student's t-test # : p <0.10 * : p <0.05 **: p <0.01
The duration 'All' data are reported only when there are 2 elements in the cell

Appendix 25 In-depth summary of mean changes in hemoglobin concentration from baseline to minimum value in placebo-controlled studies, safety population

Mean change from baseline for Hemoglobin g/dL - change from BL to minimum value up to end of treatment + 1 day 22MAY01

		100		250-500		1000-1500		2000		All	
		B	P	B	P	B	P	B	P	B	P
PAH	2-12W							-3.06 N=4 -2.44	-0.63 N=1	-3.06 N=4 -2.44	-0.63 N=1
	> 12W			-1.57 N=161 -1.09 **	-0.48 N=79					-1.57 N=161 -1.09 **	-0.48 N=79
	All									-1.60 N=165 -1.12	-0.48 N=80
CHF	2-12W							-1.57 N=43 -0.75 **	-0.82 N=20	-1.57 N=43 -0.75 **	-0.82 N=20
	> 12W					-1.52 N=208 -0.89 **	-0.62 N=116			-1.52 N=208 -0.89 **	-0.62 N=116
	All									-1.53 N=251 -0.87	-0.65 N=136
HTN	2-12W	-0.65 N=48 -0.38 *	-0.27 N=46	-0.57 N=45 -0.30 *	-0.27 N=46	-0.65 N=44 -0.38 *	-0.27 N=46	-0.96 N=48 -0.69 **	-0.27 N=46	-0.71 N=185 -0.44 **	-0.27 N=46
	> 12W										
	All										
SAH	2-12W					-2.03 N=17 -0.88	-1.15 N=7			-2.03 N=17 -0.88	-1.15 N=7
	> 12W										
	All										
All	2-12W	-0.65 N=48 -0.38	-0.27 N=46	-0.57 N=45 -0.30	-0.27 N=46	-1.03 N=61 -0.64	-0.39 N=53	-1.33 N=95 -0.89	-0.44 N=67	-0.99 N=249 -0.48	-0.51 N=74
	> 12W			-1.57 N=161 -1.09	-0.48 N=79	-1.52 N=208 -0.89	-0.62 N=116			-1.54 N=369 -0.97	-0.56 N=195
	All			-1.35 N=206 -0.95	-0.40 N=125	-1.41 N=269 -0.86	-0.55 N=169			-1.32 N=618 -0.77	-0.55 N=269

Student's t-test # : p <0.10 * : p <0.05 **: p <0.01
The duration 'All' data are reported only when there are 2 elements in the cell

Appendix 26 In-depth summary of the incidence of a decrease in hemoglobin of ≥ 1.0 g/dl (change from baseline to treatment end) in placebo-controlled studies, safety population

Hemoglobin decrease >=1.0 g/dL - from BL to end of treatment + 1 day 22MAY01

		100		250-500		1000-1500		2000		All	
		B	P	B	P	B	P	B	P	B	P
PAH	2-12W							75.0% 3/ 4 +75.0%	0.0% 0/ 1	75.0% 3/ 4 +75.0%	0.0% 0/ 1
	> 12W			65.2%105/161 +34.8% **	30.4% 24/ 79					65.2%105/161 +34.8% **	30.4% 24/ 79
	All									65.5%108/165 +35.5% **	30.0% 24/ 80
CHF	2-12W							83.7% 36/ 43 +48.7% **	35.0% 7/ 20	83.7% 36/ 43 +48.7% **	35.0% 7/ 20
	> 12W					64.9%135/208 +33.9% **	31.0% 36/116			64.9%135/208 +33.9% **	31.0% 36/116
	All									68.1%171/251 +36.5% **	31.6% 43/136
HTN	2-12W	25.0% 12/ 48 +7.6%	17.4% 8/ 46	26.7% 12/ 45 +9.3%	17.4% 8/ 46	25.0% 11/ 44 +7.6%	17.4% 8/ 46	47.9% 23/ 48 +30.5% **	17.4% 8/ 46	31.4% 58/185 +14.0% #	17.4% 8/ 46
	> 12W										
	All										
SAH	2-12W					82.4% 14/ 17 +39.5%	42.9% 3/ 7			82.4% 14/ 17 +39.5%	42.9% 3/ 7
	> 12W										
	All										
All	2-12W	25.0% 12/ 48 +7.6%	17.4% 8/ 46	26.7% 12/ 45 +9.3%	17.4% 8/ 46	41.0% 25/ 61 +20.2% *	20.8% 11/ 53	65.3% 62/ 95 +42.9% **	22.4% 15/ 67	44.6%111/249 +20.3% **	24.3% 18/ 74
	> 12W			65.2%105/161 +34.8% **	30.4% 24/ 79	64.9%135/208 +33.9% **	31.0% 36/116			65.0%240/369 +34.3% **	30.8% 60/195
	All			56.8%117/206 +31.2% **	25.6% 32/125	59.5%160/269 +31.7% **	27.8% 47/169			56.8%351/618 +27.8% **	29.0% 78/269

Fisher's exact test # : p <0.10 * : p <0.05 **: p <0.01
The duration 'All' data are reported only when there are 2 elements in the cell

Appendix 27 In-depth summary of the incidence of a decrease in hemoglobin to below the LLN in placebo-controlled studies, safety population

Hemoglobin LL or L - from BL to end of treatment + 1 day 22MAY01

		100 B	100 P	250-500 B	250-500 P	1000-1500 B	1000-1500 P	2000 B	2000 P	All B	All P
PAH	2-12W							50.0% 2/ 4 +50.0%	0.0% 0/ 1	50.0% 2/ 4 +50.0%	0.0% 0/ 1
	> 12W			22.7% 37/163 +13.8% **	8.9% 7/ 79					22.7% 37/163 +13.8% **	8.9% 7/ 79
	All									23.4% 39/167 +14.6% **	8.8% 7/ 80
CHF	2-12W							46.5% 20/ 43 +16.5%	30.0% 6/ 20	46.5% 20/ 43 +16.5%	30.0% 6/ 20
	> 12W					54.3% 114/210 +13.3% *	41.0% 48/117			54.3% 114/210 +13.3% *	41.0% 48/117
	All									53.0% 134/253 +13.5% *	39.4% 54/137
HTN	2-12W	4.2% 2/ 48 +2.0%	2.2% 1/ 46	4.4% 2/ 45 +2.3%	2.2% 1/ 46	2.3% 1/ 44 +0.1%	2.2% 1/ 46	10.4% 5/ 48 +8.2%	2.2% 1/ 46	5.4% 10/185 +3.2%	2.2% 1/ 46
	> 12W										
	All										
SAH	2-12W					94.1% 16/ 17 +37.0% #	57.1% 4/ 7			94.1% 16/ 17 +37.0% #	57.1% 4/ 7
	> 12W										
	All										
All	2-12W	4.2% 2/ 48 +2.0%	2.2% 1/ 46	4.4% 2/ 45 +2.3%	2.2% 1/ 46	27.9% 17/ 61 +18.4% *	9.4% 5/ 53	28.4% 27/ 95 +18.0% **	10.4% 7/ 67	19.3% 48/249 +4.4%	14.9% 11/ 74
	> 12W			22.7% 37/163 +13.8% **	8.9% 7/ 79	54.3% 114/210 +13.3% *	41.0% 48/117			40.5% 151/373 +12.4% **	28.1% 55/196
	All			18.8% 39/208 +12.4% **	6.4% 8/125	48.3% 131/271 +17.2% **	31.2% 53/170			32.0% 199/622 +7.5% *	24.4% 66/270

Fisher's exact test # : p <0.10 * : p <0.05 **: p <0.01
The duration 'All' data are reported only when there are 2 elements in the cell
LLN = lower limit of normal.

Appendix 28 In-depth summary of the incidence of a marked decrease in hemoglobin (LL) in placebo-controlled studies. safety population

Hemoglobin LL 17MAY01

		100 B	100 P	250-500 B	250-500 P	1000-1500 B	1000-1500 P	2000 B	2000 P	All B	All P
PAH	2-12W							25.0% 1/ 4 +25.0%	0.0% 0/ 1	25.0% 1/ 4 +25.0%	0.0% 0/ 1
	> 12W			3.0% 5/164 +1.8%	1.3% 1/ 79					3.0% 5/164 +1.8%	1.3% 1/ 79
	All									3.6% 6/168 +2.3%	1.3% 1/ 80
CHF	2-12W							6.4% 3/ 47 +1.4%	5.0% 1/ 20	6.4% 3/ 47 +1.4%	5.0% 1/ 20
	> 12W					9.1% 20/220 +5.7% #	3.4% 4/118			9.1% 20/220 +5.7% #	3.4% 4/118
	All									8.6% 23/267 +5.0% #	3.6% 5/138
HTN	2-12W	0.0% 0/ 48 0.0%	0.0% 0/ 46	0.0% 0/ 45 0.0%	0.0% 0/ 46	0.0% 0/ 44 0.0%	0.0% 0/ 46	0.0% 0/ 49 0.0%	0.0% 0/ 46	0.0% 0/186 0.0%	0.0% 0/ 46
	> 12W										
	All										
SAH	2-12W					36.8% 7/ 19 +24.3%	12.5% 1/ 8			36.8% 7/ 19 +24.3%	12.5% 1/ 8
	> 12W										
	All										
All	2-12W	0.0% 0/ 48 0.0%	0.0% 0/ 46	0.0% 0/ 45 0.0%	0.0% 0/ 46	11.1% 7/ 63 +9.3% #	1.9% 1/ 54	4.0% 4/100 +2.5%	1.5% 1/ 67	4.3% 11/256 +1.6%	2.7% 2/ 75
	> 12W			3.0% 5/164 +1.8%	1.3% 1/ 79	9.1% 20/220 +5.7% #	3.4% 4/118			6.5% 25/384 +4.0% *	2.5% 5/197
	All			2.4% 5/209 +1.6%	0.8% 1/125	9.5% 27/283 +6.6% **	2.9% 5/172			5.6% 36/640 +3.1% #	2.6% 7/272

Fisher's exact test # : p <0.10 * : p <0.05 **: p <0.01
The duration 'All' data are reported only when there are 2 elements in the cell

Appendix 29 In-depth summary of the incidence of a marked decrease in hemoglobin to < 10 g/dl in placebo-controlled studies, safety population

Hemoglobin < 10 AND LL 17MAY01

		100 B	100 P	250-500 B	250-500 P	1000-1500 B	1000-1500 P	2000 B	2000 P	All B	All P
PAH	2-12W							0.0% 0/ 4 0.0%	0/ 1 0.0%	0.0% 0/ 4 0.0%	0/ 1 0.0%
	> 12W			2.4% 4/164 1.3% +1.2%	1/ 79					2.4% 4/164 1.3% +1.2%	1/ 79
	All									2.4% 4/168 1.3% +1.1%	1/ 80
CHF	2-12W							0.0% 0/ 47 0.0%	0/ 20 0.0%	0.0% 0/ 47 0.0%	0/ 20 0.0%
	> 12W					3.2% 7/220 3.4% -0.2%	4/118			3.2% 7/220 3.4% -0.2%	4/118
	All									2.6% 7/267 2.9% -0.3%	4/138
HTN	2-12W	0.0% 0/ 48 0.0%	0/ 46 0.0%	0.0% 0/ 45 0.0%	0/ 46 0.0%	0.0% 0/ 44 0.0%	0/ 46 0.0%	0.0% 0/ 49 0.0%	0/ 46 0.0%	0.0% 0/186 0.0%	0/ 46 0.0%
	> 12W										
	All										
SAH	2-12W					15.8% 3/ 19 12.5% +3.3%	1/ 8			15.8% 3/ 19 12.5% +3.3%	1/ 8
	> 12W										
	All										
All	2-12W	0.0% 0/ 48 0.0%	0/ 46 0.0%	0.0% 0/ 45 0.0%	0/ 46 0.0%	4.8% 3/ 63 1.9% +2.9%	1/ 54	0.0% 0/100 0.0%	0/ 67 0.0%	1.2% 3/256 1.3% -0.2%	1/ 75
	> 12W			2.4% 4/164 1.3% +1.2%	1/ 79	3.2% 7/220 3.4% -0.2%	4/118			2.9% 11/384 2.5% +0.3%	5/197
	All			1.9% 4/209 0.8% +1.1%	1/125	3.5% 10/283 2.9% +0.6%	5/172			2.2% 14/640 2.2% -0.0%	6/272

Fisher's exact test # : p <0.10 * : p <0.05 **: p <0.01
The duration 'All' data are reported only when there are 2 elements in the cell

Appendix 30 Narrative for Patient SH/C9484 10128, Studies AC-052-352 and AC-052-354 in pulmonary arterial hypertension

Patient Narrative (Patient ID: [redacted]/C9484 10128)

This 48-year-old female patient with primary pulmonary hypertension was randomized to bosentan 250 mg b.i.d. in Study AC-052-352 (BREATHE-1) and rolled over to the open label extension study (AC-052-354) after 117 days of treatment, in which she received bosentan 62.5 mg b.i.d for the first 28 days followed by 125 mg b.i.d. Relevant concomitant medications were warfarin, and salbuterol. Her previous medical history included anemia, asthma, syncope, thyroid nodule, obesity, positive antinuclear antibodies and positive hepatitis B antibody.

On entry into Study AC-052-352, the patient had a hemoglobin of 11.1 g/dl and an hematocrit of 31%. By Day 35, hemoglobin and hematocrit values decreased to 10.1 g/dl and 28%, respectively. By Day 43, the patient's hematocrit decreased to 26%, and she was transfused with 2 units of blood. At the end of the study (Day 117), her hemoglobin was 10.4 g/dl with an hematocrit of 28%. During the trial, her eosinophil count remained stable, and her bilirubin concentration was found to be slightly increased on Days 63 and 95, but not on Days 35 and 117.

A complete hematology evaluation was started during the open-label study (AC-052-354), and a diagnosis of Coombs positive hemolytic anemia was made on Day 31. In addition, she had high lactate dehydrogenase and bilirubin, low haptoglobin, an increased reticulocyte count (359×10^9/l, 10.6%) and positive urine hemosiderin. Treatment with prednisolone and folic acid was initiated, and warfarin was continued. During this period the patient experienced recurrent episodes of epistaxis, and on Day 72 she was found to have gastrointestinal hemorrhage. Her hematocrit was 21.7%. She was hospitalized and transfused with 2 units of blood. Endoscopy revealed duodenal ulcers, and warfarin was discontinued. The patient's condition improved. Bosentan treatment was discontinued on Day 81 of the open-label trial.

The combination of primary pulmonary hypertension, positive anti nuclear factor and chronic anemia, which was found to be hemolytic, suggests that this patient could have an underlying autoimmune condition such as lupus erythematosus, and further tests are being done to investigate this.